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METASTATIC CALCIFICATION PRODUCED IN DOGS BY HYPERVITAMINOSIS D AND HALIPHAGIA *

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The observation¹ of widespread metastatic calcification in a man who had ingested much vitamin D and alkaline salts during several months led to a review² of the literature concerning metastatic calcification and to experiments on dogs in an attempt to reproduce the lesions noted in the patient.¹

MATERIALS AND METHODS

Sixteen clinically healthy and mature mongrel dogs (15 males and 1 female) were employed in experiments made during 1946 and the first quarter of 1947. These animals were on a stock diet of dog chow containing 725 units of vitamin D₃ as fish liver oil and 250 units of vitamin D₂ as irradiated yeast per 500 gm. The calcium content was 2.4 per cent; the phosphorus, 1.8 per cent; the carbohydrate, 55.3 per cent; the protein, 22.7 per cent; the fat, 4.2 per cent; fiber, 4.75 per cent; and moisture, 9 per cent; according to an analysis furnished by the manufacturer, L. B. Dean and Company of Denver. Water was freely available at all times. In our kennels dogs have been kept on this ration for as long as 3 years without evidence of dietary deficiency and in a good state of health, weight, and activity. The supplemental vitamin D was in the form of ertron, an electrically activated ergosterol. The supplemental alkaline salt mixture (sal hepatica³) contained the phosphate, bicarbonate, sulfate, and chloride salts of sodium. Seven dogs received both vitamin D and the alkaline salts; 5, vitamin D alone; 2, the alkaline salts alone; and 2, the stock diet only. The

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manner of death, the number of days of each experiment, the body weight of each animal at the beginning and end of the experiments, the percentage of original body weight lost, the total international units of vitamin D₂, and the total grams of sal hepatica have been summarized in Table I. Both substances, given orally, were incorporated in small boluses of ground beef. The vitamin D was usually given in doses of 100,000 units daily, except: (a) in the first week in dogs 1 and 2 when 50,000 units daily were administered, (b) when temporary meat shortages occasionally hindered dosage for 1 to 2 days or even a week, and (c) in dogs 20 and 21 which received 150,000 units of vitamin D daily. The alkaline salts were given in doses of 7.5 to 8 gm. daily. The predominant breed in each dog used was estimated as follows: dog 1, beagle hound; dog 2, cocker spaniel; dog 7, bulldog; dog 16, poodle; dog 20, Dalmatian; and the other 11, terriers of various types (8 fox, and 1 each Scotch, wire-haired, and Airedale).

The tissues studied were obtained at autopsy immediately or within a few hours after death. The thyroid, parathyroid, and thymus glands, spleen, liver, gallbladder, pancreas, left adrenal gland, urinary bladder, prostate, testes, lymph nodes, ribs, vertebrae, and pituitary gland were fixed in Zenker's fluid. The heart, aorta, lungs, esophagus, stomach, intestines, right adrenal gland, kidneys, and brain were fixed in a 4 per cent solution of formaldehyde, so that any possible interference of potassium bichromate or mercuric chloride would be avoided in determining calcification of these viscera. The tissues were washed, trimmed, dehydrated and cleared in dioxane, imbedded in paraffin, sectioned at 8 μ , and stained with hematoxylin and eosin.

RESULTS

CLINICAL FINDINGS

All dogs given vitamin D, alone or with alkaline salts, lost 32 to 61 per cent of their original body weight. Inappetence was distinctly in evidence before each experiment was half completed and hypodipsia, apathy, and terminal coma were observed in those dogs which died. Occasionally, hematemesis or melena was noted. The dogs receiving only alkaline salts, or on the stock diet only, showed no significant changes in body weight and maintained both appetite and general activity. By the mid-period of each experiment, and usually before, the dogs on vitamin D alone exhibited small, hard, dark brown stools as contrasted with the bulky, semiliquid, unformed, light tan stools of the dogs on alkaline salts alone. Those dogs given both vitamin D and alkaline salts had moderate-sized soft, formed, light brown stools.

TABLE I
Data on Development of Costochondral Junction, Mode of Death, Number of Days of Each Experiment, Weight in Kg. at Beginning and End of Experiment, Percentage of Weight Lost, the Total Amount of Vitamin D in Millions of Units, and the Total Amount of Sal Hepatica in Gm.

| Dog | Costochondral junction | Mode of death | Days | Weight | | Weight loss per cent | Vitamin D million units | Sal hepatica gm. |
|-----|------------------------|---------------|------|------------------|------------|-------------------------|----------------------------|---------------------|
| | | | | Beginning kg. | End kg. | | | |
| 4 | Cartilage open | Died | 36 | 7.0 | 4.5 | 36 | 3.4 | 272 |
| 13 | Cartilage open | Sacrificed | 26 | 9.5 | 5.5 | 42 | 2.0 | 128 |
| 15 | Cartilage open | Died | 37 | 6.6 | 4.0 | 39 | 2.5 | 200 |
| 1 | Bony seal | Died | 74 | 11.4 | 6.7 | 41 | 6.4 | 573 |
| 2* | Bony seal | Sacrificed | 47 | 9.5 | 6.4 | 33 | 3.8 | 305 |
| 10 | Bony seal | Died | 83 | 13.2 | 5.0 | 61 | 7.6 | 570 |
| 19 | Bony seal | Died | 52 | 8.6 | 4.1 | 53 | 4.6 | 345 |
| 6 | Cartilage open | Died | 19 | 8.0 | 4.8 | 40 | 1.7 | |
| 21 | Cartilage open | Died | 30 | 11.0 | 7.5 | 32 | 4.35 | |
| 7 | Bony seal | Sacrificed | 32 | 8.0 | 4.3 | 46 | 5.1 | |
| 9 | Bony seal | Died | 54 | 10.2 | 4.5 | 56 | 5.4 | |
| 20 | Bony seal | Died | 54 | 13.0 | 6.4 | 51 | 8.1 | |
| 10 | Bony seal | Sacrificed | 65 | 8.4 | 8.6 | +2 | | |
| 11 | Bony seal | Sacrificed | 68 | 9.1 | 9.1 | 0 | | 451 |
| 8 | Bony seal | Sacrificed | 52 | 12.5 | 12.0 | 4 | | 475 |
| 12 | Bony seal | Sacrificed | 61 | 9.5 | 9.3 | 2 | | |

* Female.

All three groups had stools unlike the moderately firm, well formed, medium brown stools of the 2 control dogs and of many others on the stock diet observed in our kennels.

AUTOPSY FINDINGS

The gross features in the dogs receiving vitamin D alone or in combination with alkaline salts included severe wastage of the adipose tissues; dryness of the serous membranes; very small thymus glands, lymph nodes, and spleens; the frequent occurrence of one to three soft calcific plaques, 1 to 8 mm. in diameter, in the walls of one or more sinuses of the aortic valves; three to six flecks of soft calcium, 0.5 to 5 mm. in diameter, in the endocardium of the posterior wall of the left atrium; scanty, tan or dark red fluid in the stomach and intestines; small parathyroid glands, prostate, and testes; a thin, pale yellow line of calcium at the corticomedullary junction of the kidneys in 4 dogs; and pale tan marrow in the ribs and vertebrae of some dogs. Calcification in the lungs and stomach was not grossly visible or palpable, although the deposits in the splenic capsule of one dog and in the pulmonic arteries of 2 dogs were easily seen. The brains of all dogs were grossly normal.

Microscopic Calcification

The most important microscopic features were the calcium deposits in the endocardium of the left atrium, the walls of the aortic valvular sinuses, the walls of the alveoli and alveolar ducts of the lungs, the loops of Henle in the kidneys, and the stroma around the glands of the mucosa of the fundus of the stomach. To a certain degree, calcium casts in the renal collecting tubules were noteworthy and a few miscellaneous calcific deposits should be mentioned. All of these features have been included in Table II.

Heart. The calcific deposits in the endocardium of the left atrium first affected the elastic fibrils and then were spread out in granular aggregations (Fig. 1) and impinged on the inner aspect of the myocardium. No calcium was found in representative sections of either ventricle, the septum, or the right atrium.

Walls of Sinuses of Cusps of Aortic Valve. The intima (Fig. 2) was predominantly affected, but the media was focally involved, both layers showing elastic fibrils incrustated by calcium salts which were dispersed between them.

Lungs. The walls of the alveoli and alveolar ducts showed clearly defined plates of calcium of varying thickness (Fig. 3). When the

TABLE II
Calcification in Endocardium of Left Atrium of Heart, in Walls of Sinuses of Aortic Valve, in Alveoli and Alveolar Ducts of Lungs, in Henle's Loops of Kidneys, in Stroma of Mucosa of Stomach, in Lumen of Renal Collecting Tubules, and in Other Sites

| Dog | Left atrial endocardium | Aortic valvular sinuses | Lungs | Henle's loops | Stomach | Renal collecting tubules | Other sites and grade |
|-----|-------------------------|-------------------------|---------|---------------|---------|--------------------------|--|
| 4 | grade II | grade I | grade o | grade o | grade o | grade I | None |
| 13 | o | I | o | o | o | II | None |
| 15 | o | I | o | III | III | III | Bronchial cartilages, I |
| 1 | I | I | V | o | o | I | Splenic capsule, V; pulmonary veins and arteries and bronchial cartilages, III |
| 2 | II | I | IV | o | o | II | Pulmonary arteries and bronchial cartilages, II |
| 16 | o | III | o | IV | III | II | Pulmonary arteries, III |
| 19 | I | III | V | I | III | III | Pulmonary arteries, IV; pulmonary veins and bronchial cartilages, I |
| 6 | o | I | o | I | o | II | None |
| 21 | I | II | o | I | o | II | None |
| 7 | I | II | II | o | o | I | Bronchial cartilages, I |
| 9 | o | III | o | V | IV | I | None |
| 20 | o | o | o | III | III | III | None |
| 10 | o | o | o | o | o | I | None |
| 11 | o | o | o | o | o | II | None |
| 8 | o | o | o | o | o | I | None |
| 12 | o | o | o | o | o | I | None |

Grade I = minimal; Grade V = maximal; Grades II to IV = intermediate; o = absent.

veins were calcified, the intima was chiefly affected, but the arteries showed calcium deposits mainly in the adventitia and the outer aspect of the media in relation to elastic fibrils. Bronchial cartilages were unevenly marked by plaques of calcium, which largely obliterated both ground substance and chondrocytes. Calcium involved the intima of the pulmonic artery in dog 16 and the adventitia and outer media in dog 19. The walls of the sinuses of the pulmonic valve also showed intimal calcium deposits in dog 19.

Kidneys. So far as could be determined, the principal deposition of calcium in the kidneys was in the basement membranes, cells, and lumina of Henle's loops (Fig. 4), apparently preponderantly in the broad or ascending limbs. Occasional calcific casts were noted within the distal convoluted and connecting tubules. In dog 20 only, calcium was observed in the basement membranes of several glomerular capsules and within the corresponding capsular spaces. The finding of calcium casts in the collecting tubules of all 16 dogs was of equivocal significance, although dogs 15, 19, and 20 exhibited also calcification of the basement membranes and cells of several collecting tubules.

Stomach. The stroma around the glands of the middle third of the mucosa of the fundus was distinctly saturated with calcium (Fig. 5) in 5 dogs. The parenchymal cells rarely were calcified.

Other Sites. The splenic capsule of dog 1 was widely inundated by calcium salts, conspicuously precipitated on elastic fibrils. The medium and small arteries in all viscera examined, except those of the lungs, lacked calcium demonstrable by hematoxylin.

Other Microscopic Observations

Testes. The tubules were reduced in size and the stroma was relatively prominent. The seminal epithelium often consisted only of a layer of spermatogonia, as seen especially in dog 1 (Fig. 6) and dog 16, which lived longest. It was intact and spermiogenesis was greatly reduced only in dog 6 which survived the shortest time. Definite evidences of atrophy of the seminal epithelium in the other dogs which received vitamin D alone or with alkaline salts included the complete absence of spermia, only rare tubules with the epithelium developed to the spermatid stage, and most tubules lined by spermatogonia or by spermatogonia and varying numbers of primary and secondary spermatocytes, usually the former. The testes of both dogs on alkaline salts and of the two control dogs (Fig. 7) were normal and active. The data concerning the testes, the prostate, the costal and vertebral marrow, the lymphoid tissue, and the fat tissue have been collected in

Table III. The genitalia of the female dog, no 2, were normal and in the stage of anestrus of what was at least its second estrual cycle.

Prostate. Of the 11 male dogs on vitamin D alone or in combination with alkaline salts, the prostate gland showed advanced atrophy in 7 and partial atrophy in one. Three had incompletely developed prostates which could not be accurately evaluated for atrophy. The atrophic changes (Fig. 8) included decreased height and size of the epithelial cells of both ducts and acini, especially of the latter, with shrinkage of the cytoplasm and condensation of the nuclei. The acini and ducts were flattened and slit-like, and sometimes the lumina were practically obliterated. The stroma, also shrunken, was relatively increased as compared to the parenchymal elements. These atrophic changes were clearly in contrast to the normal state (Fig. 9).

Ribs. In order to correlate skeletal and genital growth, special attention was paid to the development of the costochondral junctions, two of which from each dog were examined histologically after decalcification in 5 per cent nitric acid. As indicated in Table I, dogs 4, 13, 15, 6, and 21 showed an open line along the proximal edge of the cartilage (Fig. 10), whereas all other dogs, including the 2 control animals, showed a bony seal along this edge of the cartilage whether the bony cortex of the distal end of the rib was incomplete (Fig. 11) or complete (Fig. 12). The implications of this will be discussed. To supplement these observations, the prostate, testes, and costochondral junctions of 15 other dogs, normal mature males, have been studied. Twelve showed a bony seal at the proximal edge of the cartilage of the costochondral junction and had normal testes, but only 11 had normal prostate glands, the twelfth dog having a partly developed prostate. The other 3 had open cartilage at the costochondral junction and normal prostate and testes. Since genital maturity precedes skeletal maturity by at least 10 years in man, as demonstrated by cessation of growth at the costochondral junctions, the supposition that a similar relationship holds in the dog is not unreasonable, allowing for the difference in the life spans of the two species. From the evidence noted in the 15 additional control male dogs so far examined, the finding of a bony seal at the cartilage of the costochondral junctions of a dog indicates that his chances of having normal and active testes would be 100 per cent and of having a normal and fully developed prostate would be well over 90 per cent.

Parathyroid Glands. The parathyroid glands of dogs receiving vitamin D alone or in combination with alkaline salts were smaller than normal glands. The cells were packed more closely, showed even less

distinct cytoplasmic borders than the usually hazy outlines, and contained contracted nuclei with condensed chromatin and nucleoli which were often obscured (Figs. 13 and 14). The stroma and blood vessels were relatively more conspicuous. Measurements by a standardized micrometer ocular have been made on 50 representative nuclei of the parathyroid glands of each of 30 normal dogs. As shown in Table IV, the parathyroid nuclei of the dogs receiving vitamin D alone or in combination with alkaline salts were significantly smaller than those of the 30 normal dogs.

TABLE IV

Statistical Comparison between the Size of the Nuclei of Parathyroid Glands in 30 Normal Dogs and in 12 Dogs Given Large Doses of Vitamin D

| | Range | Sum of values | Mean | Sum of squares of values | Sigma | Standard error of mean |
|-------------------|------------------|---------------|--------------|--------------------------|-------|------------------------|
| Normal (30) | μ 5.8-6.5 | 1855 | μ 6.2 | 114803 | 0.20 | 0.0365 |
| Experimental (12) | 4.3-5.7 | 640 | 5.3 | 34302 | 0.37 | 0.1068 |

Standard error of difference = 0.113.
Statistical significance = 7.5.

Bone Marrow. The bone marrow was studied qualitatively by Giemsa staining. For the 12 dogs on vitamin D alone or in combination with alkaline salts, the marrow cell/fat cell ratio was reduced in 8 and increased in 2. The 2 sacrificed, which showed this ratio within normal limits, might have demonstrated a reduction had they died spontaneously. The myeloid/erythroid ratio was increased in 10 of 12 dogs, decreased in one, and normal in one. In the 10 animals with an increased myeloid/erythroid ratio, the neutrophilic granulocyte line was within normal limits in respect to composition, or was moderately shifted to the left. Eight of the 12 dogs showed atrophic fat cells in the bone marrow and 4 exhibited normal fat cells. The fat cells showed shrinkage, loss of lipids, and increasing trend to a central position of their nuclei. The space which they formerly occupied was filled by basophilic fluid, fine granules, or filaments. The vertebral marrow of an experimental dog so affected (Fig. 15) contrasted strikingly with that of a control dog (Fig. 16).

Bones. The ribs and vertebrae were studied routinely after decalcification in 5 per cent nitric acid. Dogs 9 and 20 displayed well developed osteoporosis (Fig. 17) in the vertebrae as well as in the ribs. This consisted of widening of Volkmann's and haversian canals, filling of them by increased marrow, both red and yellow, a disappearance of

osteocytes from the bone at the margins of these canals, a peeling away of granular calcium and fragmented lamellae of damaged bone, a thinning of the cortex, and a widening of marrow spaces in the medulla. Osteoclasts were absent.

Qualitative Chemical Analysis of Calcium Deposits. The more abundant deposits, which were seen microscopically in sections stained with hematoxylin and eosin as dark blue granular or homogeneous masses and plates, were presumed to be calcium salts. These were analyzed by qualitative tests previously employed.¹ The walls of the aortic sinuses in dogs 16, 19, and 9, the lungs of dogs 1, 2, and 19, the kidneys of dogs 15, 16, 9, and 20, the gastric mucosa of dogs 15, 16, 19, 9, and 20, the splenic capsule of dog 1, and the pulmonic artery of dog 19 all contained calcium phosphate by these tests. Carbonate was absent from all of these deposits. Especially interesting was the negative reaction for carbonates in the lungs of dogs 1, 2, and 19, since the patient described¹ had only calcium carbonate in his lungs.

COMMENT

The choice of an activated ergosterol preparation as the source of vitamin D in these experiments was determined by the ready availability of capsules, each containing 50,000 international units of the vitamin. The employment of doses of the magnitude of 100,000 to 150,000 units daily might seem a waste of the vitamin, but determinations⁴ of the intestinal excretion by dogs after a single massive dose of an irradiated ergosterol preparation demonstrated that the vitamin persisted in the feces for several months. Also, determinations of the serum levels of vitamin D in six human subjects receiving large doses⁵ over several months indicated that several weeks were required for the highest serum level of the vitamin to be attained and suggested that the vitamin as D₂, because of the presence of an unsaturated bond,⁶ is absorbed quite efficiently under normal conditions of alimentation until and after this maximal level is reached. Other experiments^{7,8} have showed the efficacy of vitamin D of plant origin (D₂), as compared with that of animal origin (D₃), in causing calcification of soft tissues. With irradiated ergosterol and tuna liver oil, both at various dosage levels,⁷ calcification was found in the kidneys, heart, stomach, lungs, and aorta of rats, but the calcium deposits were much heavier when irradiated ergosterol was given, indicating that vitamin D₂ is more efficacious unit for unit than vitamin D₃ in causing soft tissue calcification. With both forms of vitamin D, the kidneys were the organs most involved by calcific deposits, an observation described

by several investigators.² Another study⁸ on rats demonstrated that depression of growth, mortality, hypercalcemia, and visceral calcification were much more severe in the animals getting high levels of irradiated ergosterol than in those on comparable doses of fish liver oil concentrate.

Much has been written⁹ with regard to the rôle of vitamin D in the absorption of calcium and phosphorus from the intestinal tract. Some workers have shown that the presence of vitamin D in the intestinal tract causes increased absorption of calcium and little effect on the absorption of phosphorus. Other evidence¹⁰ has indicated that the presence of the phosphate radical potentiates the absorption of vitamin D from the intestinal tract. An observation supporting the same conclusion was the increase of tissue calcification obtained by vitamin D when a high phosphorus diet was given.¹¹ These observations,^{10,11} together with the enhancement of soft tissue calcification by alkaline diets¹² and by the intravenous injection of sodium bicarbonate solution,¹³ indicate that the employment of alkaline salts in the experiments described in the present paper would be efficacious in furnishing both a high level of phosphate ions to enhance absorption of vitamin D from the intestine and also alkaline salts to augment soft tissue calcification. Several other combinations of alkaline salts which might be similarly employed are readily and commercially available. In contrast, alkaline diets alone result in relatively little soft tissue calcification.² A high calcium diet magnifies⁶ the action of vitamin D in the healing of rickets and in the production of soft tissue calcification, although this varies with the conditions of the experiment.

The intermediate products formed from the ultraviolet irradiation of ergosterol in the production of vitamin D₂, or calciferol, as well as the products formed from the overirradiation of calciferol were thought⁶ to be solely responsible for the toxic changes and tissue calcification when irradiated ergosterol was first used experimentally, while the antirachitic properties only were attributed to the calciferol. Others have demonstrated that the toxic-calcific and antirachitic activities of vitamin D₂ obtained from irradiated ergosterol are parallel, and that other products formed in the overirradiation or overheating of ergosterol were apparently harmless¹⁴ when given in the same doses and for the same length of time. The antirachitic effects of irradiated ergosterol do not come into consideration, since the dogs used in the present experiments showed neither clinical nor pathologic signs of rickets.

Several observations may be made concerning the data contained in Tables I to III. The smaller total amount of calcification in the tis-

sues of dogs 4, 13, 15, 6, and 21 with open cartilage at their costochondral junctions contrasted rather sharply with the more extensive calcification in dogs 1, 2, 16, 19, 7, 9, and 20 with bony seals at the cartilage of the costochondral junctions. This could be due to the continuing demands by the unossified cartilage of the costochondral junctions for vitamin D, so that the substance would not be entirely free, although present in excess, for the calcification of soft tissues. The presence of new-formed bony spicules in the medulla of the ribs at the costochondral junctions with open cartilage would lend support to this hypothesis, since new medullary bone was not present when a bony seal was found at the costochondral junctions.

The prime function of vitamin D is to exert its effects at areas of ossifying cartilage.⁹ The tissues of dogs on vitamin D and alkaline salts showed a greater total amount of calcification than those of the dogs on vitamin D alone. The dogs on both vitamin D and alkaline salts also had much more calcium deposited in the lungs than those on vitamin D alone. Dogs receiving both sodium bicarbonate solution intravenously and vitamin D showed increased pulmonary as well as renal calcification by quantitative analysis.¹³ The patient described previously¹ had a tremendous amount of calcium in the lungs as compared with that found in his kidneys. This finding contrasted with the large amount of calcium noted in the kidneys of 8 other patients² who received large amounts of vitamin D. The calcification of the basement membranes and cells and the calcific casts in the lumina of the proximal one-third of the ascending limbs of the loops of Henle in the kidneys in dogs 15, 16, 19, 6, 21, 9, and 20 were observed by Goormaghtigh and Handovsky¹⁵ in dogs getting large doses of vitamin D₂. They also found calcific casts in the lumina of the distal convoluted, connecting, and collecting tubules.

Harrison and Harrison¹⁶ have found, more pronounced in young dogs, a great increase in the tubular reabsorption of phosphate when vitamin D was given. With this increased absorption of phosphate and a high level of calcium ions in the urine being concentrated, the loops of Henle of the dog would thus be especially vulnerable to calcification as indicated by the anatomic evidence in the present and previous¹⁵ experiments.

Rats, which have a similar renal structure to dogs, placed on high phosphate diets by McFarlane¹⁷ and Oliver,¹⁸ developed calcification of the cells of the ascending limbs of Henle's loops and of the cells of the ends of the proximal convoluted tubules, showing the importance of increased phosphate in the diet in causing renal calcification. The presence of calcific casts in the renal collecting tubules of the dogs

showing also calcification of Henle's loops may be explained on the basis of calcified cells being sloughed in aggregates from these loops and carried by the urine distally. Even in dogs 4, 13, 1, 2, and 7, in which calcium was not observed in Henle's loops, calcification of single cells in these loops may have been too minimal to be detected microscopically, but the calcified cells conglomerated into casts were visible within the collecting tubules. Similarly, the 2 dogs on alkaline salts alone could have had these casts in the same manner through increased phosphate furnished by the sodium phosphate in the mixture. The only factor possibly responsible for these casts in the 2 control dogs was the relatively low calcium/phosphorus ratio, 1.33, in the stock diet, since a 2:1 ratio approaches the ideal more closely.⁹ Casts in the renal collecting tubules have not been observed in dogs on the stock diet and used in other experiments in this laboratory. Leptospirosis was not found in any dog in the present series.

In connection with the localization of calcium deposits in the loops of Henle in these experiments, the sections of kidneys of female dogs with bile fistulas, which had been given large doses of estrone, were reviewed. The lipid deposits in these kidneys were originally thought¹⁹ to be located in the cells of the cortical portions of the collecting tubules, but now have been more accurately localized in the cells of the distal two-thirds of the ascending limbs of the loops of Henle.

Other factors favoring metastatic calcification in the tissues, including the heart, aorta, lungs, kidneys, stomach, and the miscellaneous sites summarized in Table II, have been discussed.²

The high degree of atrophy of the testes in the dogs receiving vitamin D alone or in combination with alkaline salts might be properly credited to the severe inanition noted in the last days of the experiment and at autopsy. Certainly, the atrophy of the lymphoid (thymus, spleen, lymph nodes, intestines, penile sheath) and adipose tissues could be explained on this basis. The possibility that there is a specific effect of vitamin D₂ through the stilbenoid linkage²⁰ present in its structure, similar to that in stilbestrol,²¹ must be considered. The same reasoning might also hold for the atrophy of the prostate gland seen in dogs 4, 15, 1, 19, 7, 9, and 20, although squamous metaplasia of ducts and acini caused by natural and synthetic estrogens, notably stilbestrol, was not observed. Harris and Moore¹⁴ found that male and female rats placed together for several months on a diet containing 15 per cent cod liver oil failed to reproduce, but exact reasons for this phenomenon were not given. The patient¹ on vitamin D and alkaline salts showed aspermiogenesis.

Atrophy of the parathyroid glands in the dogs on vitamin D alone

or with alkaline salts was apparently due to the vitamin itself. With hypercalcemia and hyperphosphatemia resulting from hypervitaminosis D as previously discussed,² the demands for parathyroid hormone are diminished, since the parathyroid glands are not called upon to maintain the blood levels of calcium and phosphorus by the normal degree of activity. The situation is different from that in secondary hyperparathyroidism in which the kidneys have been severely damaged first and the parathyroid glands are then stimulated to overwork hyperplasia to aid in the excretion of excessive phosphate in the blood and by their overactivity later to raise the level of blood calcium. In hypervitaminosis D, increased levels of both blood calcium and blood phosphorus are thrust upon normal parathyroid glands, as well as upon normal kidneys, so that the glands undergo atrophy of disuse. No study of parathyroid glands of animals receiving large doses of vitamin D has been found to date, so that the observations reported will require confirmation.

The decrease of the marrow cell/fat cell ratio in the bone marrow of dogs 4, 15, 1, 16, 21, 7, 9, and 20 indicates either a direct effect of vitamin D or of the inanition resulting from its ingestion in large doses. The increased myeloid/erythroid ratio, also noted in the human patient,¹ in all animals on D alone or in combination with alkaline salts except dogs 16 and 19, might be interpreted as a specific effect of the stilbenoid linkage in vitamin D₂, since the first effect of stilbestrol on the bone marrow of dogs is to stimulate strongly neutrophilic granulocytopoiesis as has been indicated.¹⁰ Bronchopneumonia, widespread in dog 21 and early in dog 20, and acute focal cystitis in dog 6, probably contributed to this neutrophilic granulocytopoiesis, but infection was not found in the other 7 dogs concerned. The atrophic fat cells in the bone marrow of dogs 4, 15, 1, 16, 19, 7, 9, and 20 were directly related to the severity of the percentage of original body weight lost, since these animals lost 36 to 61 per cent as compared to dogs 13, 2, 6, and 21 which had normal fat cells and lost between 32 and 42 per cent. The atrophic fat cells in the bone marrow probably represented a fat depot depleted very late in severe inanition. The atrophic fat cells and the serous fluid in the spaces formerly occupied by normal fat cells constitute the condition of serous atrophy of the fatty bone marrow, a term much to be preferred to "gelatinous degeneration."

Osteoporosis was seen, conspicuously in the vertebrae, only in dogs 9 and 20, which were given vitamin D alone for shorter periods than those during which dogs 1 and 16 received both vitamin D and alkaline salts. This might indicate a sparing action of an alkaline diet in respect

to visible destructive skeletal changes. Perhaps a lower daily dose of vitamin D over a longer period of time might result in more profound skeletal changes in dogs whether this substance be given alone or with alkaline salts. In rats on vitamin D over a relatively long time bony changes were striking,²² especially in the cortical bone of the diaphysis.

SUMMARY

1. Seven dogs receiving vitamin D and a mixture of alkaline salts and 5 given vitamin D alone showed inappetence, a loss of 32 to 61 per cent of original body weight, hypodipsia, and apathy. Two dogs on alkaline salts and 2 control dogs did not exhibit these features. The dogs in each of the four groups showed characteristic stools.

2. All dogs receiving vitamin D alone or in combination with alkaline salts revealed varying degrees of calcification in the endocardium of the left atrium, the walls of the aortic sinuses, the alveoli and alveolar ducts of the lungs, the mucosa of the fundus of the stomach, the loops of Henle of the kidneys, and miscellaneous sites. Five dogs with open cartilage at the costochondral junctions showed less total soft tissue calcification than the 7 dogs with bony seals at their costochondral junctions. The soft tissues of the 7 dogs on both vitamin D and alkaline salts revealed more calcification than those of dogs on vitamin D alone. This was true especially in the lungs. The morphologic characteristics of calcium in the kidneys were correlated with the physiologic mechanisms concerned. By qualitative chemical analysis calcium phosphate was found in the more heavily calcified sites.

3. Atrophy of the testes, prostate gland, parathyroid glands, bone marrow, and of the lymphoid and adipose tissues was discussed in relation to possible direct and indirect effects of vitamin D.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 85

- FIG. 1. Dog 4. Calcium deposits in endocardium of left atrium. Single elastic fibrils involved in lower half. Abundant calcium impinged on inner aspect of myocardium in upper half. Lumen at right. $\times 50$.
- FIG. 2. Dog 15. Deposition of calcium salts, mainly in intima of wall of aortic sinus. Lumen at right. $\times 50$.
- FIG. 3. Dog 1. Lung. Plates of calcium in walls of several alveoli and in wall of alveolar ducts in upper left-hand corner. $\times 125$.
- FIG. 4. Dog 9. Calcification in Henle's loops of kidney. Inner margin of cortex at top. Base of pyramid at bottom. $\times 35$.
- FIG. 5. Dog 15. Calcium deposits in stroma of middle third of fundic portion of gastric mucosa. $\times 50$.
- FIG. 6. Dog 1. Atrophic tubules in testis. Entire tubule lined by spermatogonia in upper half. $\times 175$.
- FIG. 7. Dog 8. Normal testis. Well developed seminal epithelium and numerous spermia in a tubule, less than one-half of the length of which is depicted. $\times 175$.

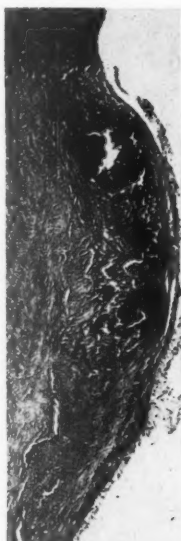
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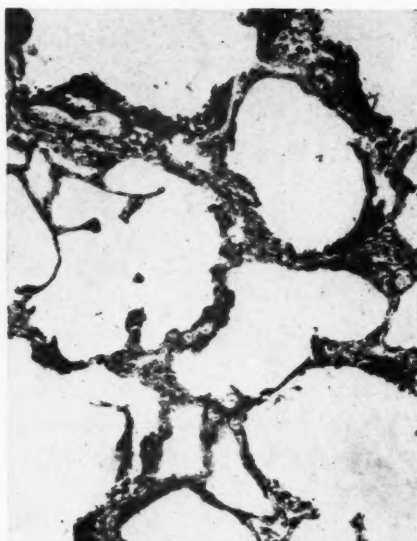
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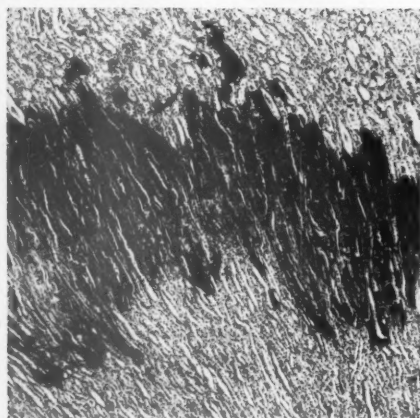
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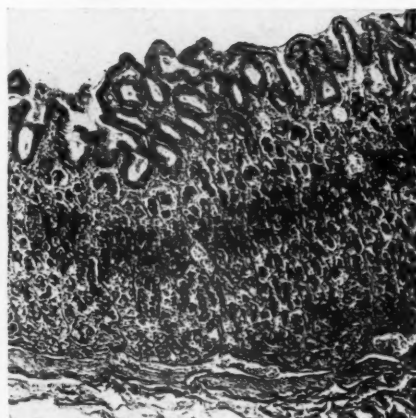
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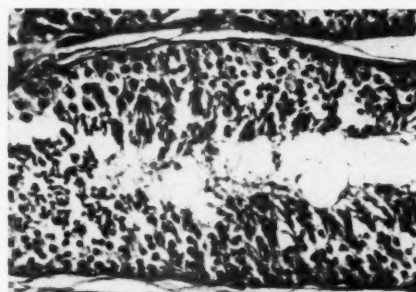
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Mulligan and Stricker

Calcification Produced by Hypervitaminosis D

PLATE 86

FIG. 8. Dog. 1. Atrophic prostate. $\times 50$.

FIG. 9. Dog 8. Normal prostate. $\times 50$.

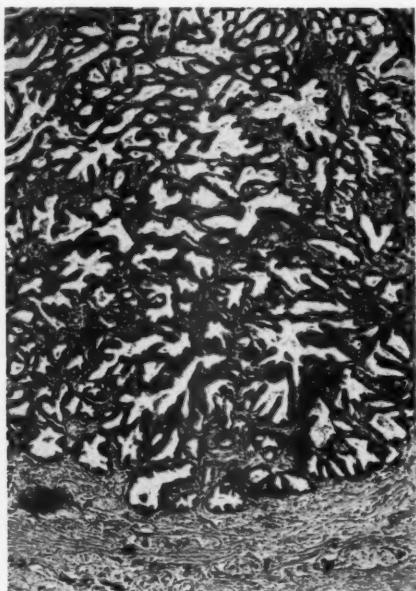
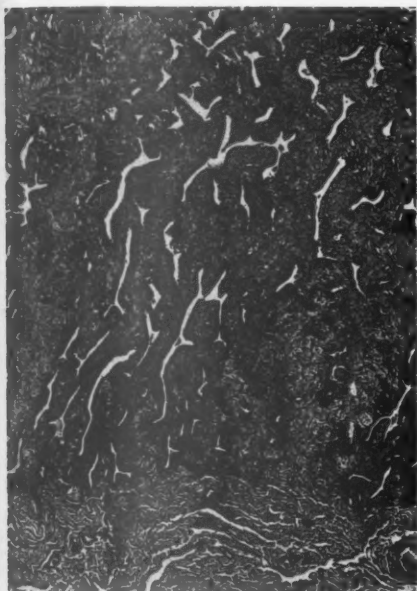
FIG. 10. Dog 6. Rib, longitudinal section. Open cartilage, new-formed medullary bony trabeculae, and incomplete cortex at costochondral junction. $\times 35$.

FIG. 11. Dog 16. Rib, longitudinal section. Bony seal along cartilage and incomplete cortex at costochondral junction. Serous atrophy of fatty marrow. $\times 35$.

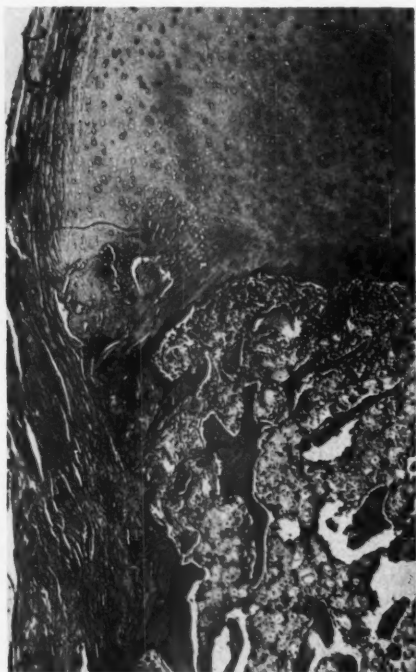
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Mulligan and Stricker

Calcification Produced by Hypervitaminosis D

PLATE 87

FIG. 12. Dog 11. Rib, longitudinal section. Bony seal along cartilage and complete cortex at costochondral junction. $\times 35$.

FIG. 13. Dog 16. Atrophic cells in parathyroid gland. Nuclei shrunken and stroma and blood vessels prominent. $\times 600$.

FIG. 14. Dog 8. Normal parathyroid gland. $\times 600$.

FIG. 15. Dog 16. Vertebral marrow. Reduction of marrow cells. Atrophic fat cells, one well seen in lower left-hand corner. $\times 450$.

FIG. 16. Dog 8. Normal vertebral marrow. Fat cell in lower right-hand corner. Megakaryocyte at left of center. Numerous marrow cells. $\times 450$.

FIG. 17. Dog 9. Osteoporosis, vertebra. Widening of Volkmann's and haversian canals. Fragmented calcium in dark, irregular bands in lower edge. Widened marrow spaces and peeling away of bony lamellae. $\times 50$.

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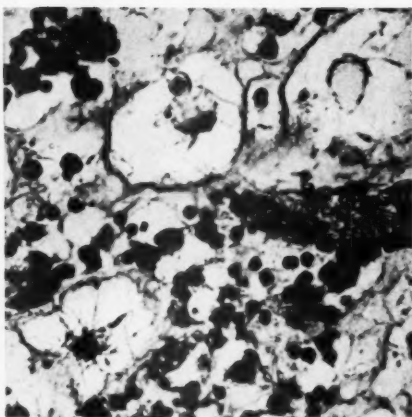
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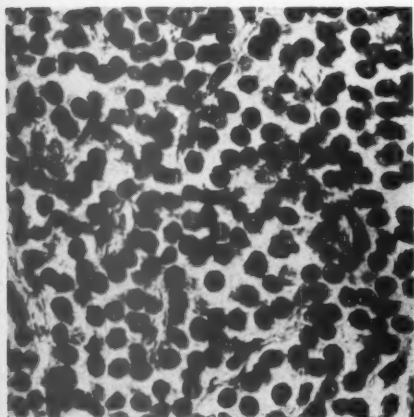
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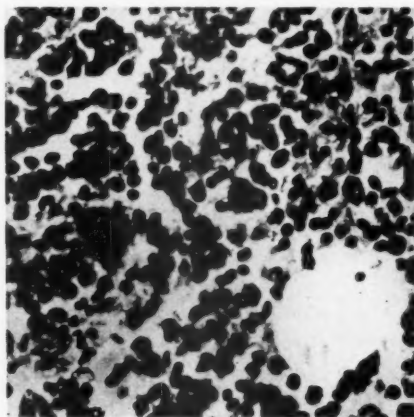
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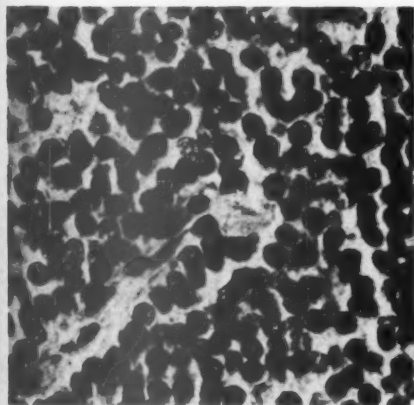
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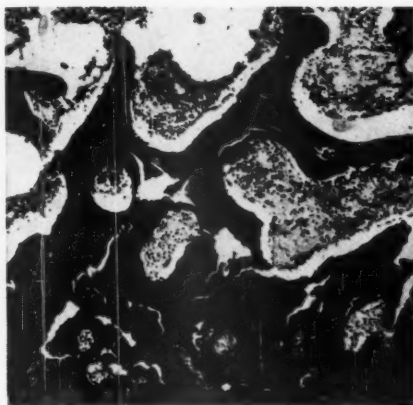
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Mulligan and Stricker

Calcification Produced by Hypervitaminosis D

CICATRIZING ENTERITIS (REGIONAL ILEITIS) AS A PATHOLOGIC ENTITY

ANALYSIS OF ONE HUNDRED AND TWENTY CASES *

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Cicatrizing enteritis is a relatively uncommon¹ nonspecific inflammatory disease, usually diagnosed by exclusion of tuberculosis, syphilis, actinomycosis, and other bowel infections of specific etiology. In the voluminous literature the pathologic observations often are relegated to fine print. It is the purpose of the present article to emphasize the pathologic features, and especially the developmental stages, of cicatrizing enteritis.

HISTORICAL ASPECT

What is now called cicatrizing enteritis or regional ileitis has been described under various names for over a century. The earliest case recorded was in 1806.^{2,3} Until relatively recently there have been only sporadic case reports. Braun,⁴ in 1909, described 7 cases with inflammatory masses in the colon which had the appearance of neoplasm. These were not associated with diverticula and the cause of the inflammation could not be discovered. In 1913 Dalziel⁵ described as "chronic interstitial enteritis" 9 cases characterized by thickening of the small or large intestines. Tietze⁶ included some similar cases in an encyclopedic article on inflammatory intestinal masses. Låwen⁷ observed an unusual condition which he named "Appendicitis fibroplastica," but later⁸ recognized as a variation of cicatrizing enteritis.

Moschcowitz and Wilensky⁹ were the first to point out that many cases of nonspecific granulomas of the intestines had been diagnosed incorrectly as hyperplastic tuberculosis. The use of stricter criteria for diagnosis of tuberculosis, it was rightly predicted, would reduce the number of such cases. Mock¹⁰ provided confirmatory evidence of the nonspecificity of many of the cases called tuberculosis. He also considered that cicatrizing enteritis should be diagnosed by exclusion of inflammatory reactions of known cause. In the Continental literature,¹¹⁻¹⁵ the disease has been called "ileitis stenosans" or "ileitis ulcerosa" and emphasis placed on the diverse kinds of injury and irritation which can lead to inflammatory intestinal stenosis.

*Received for publication, May 21, 1947.

The classic article by Crohn, Ginzburg, and Oppenheimer,¹⁶ in 1932, renamed and defined regional ileitis so well that now, 15 years later, their statements would require few alterations. Recognition of more widespread intestinal involvement,¹⁷⁻¹⁹ however, has led to proposals of inclusive terms such as cicatrizing enteritis^{20,21} and regional enteritis,²² and many others.²³⁻²⁷

TABLE I
Gross Features of Cicatrizing Enteritis in 120 Cases (Male, 60; Female, 60)

| Location | Number of cases | Percentage |
|-----------------------|-----------------|------------|
| Ileum | 112 | 93 |
| Appendix | | 24 |
| Proximal colon | 18 | 15 |
| "Skip areas" | 11 | 9 |
| Jejunum | 5 | 4 |
| Distal colon | 1 | |
| Previous appendectomy | | 37 |
| Meckel's diverticulum | 4 | |
| Other diverticula | 4 | |

| Measurements of involved area | Minimum | Maximum | Average |
|-------------------------------|------------|--------------|-------------|
| Affected length | cm. 1.5 | cm. 150.0 | cm. 24.0 |
| Thickness of intestinal wall | 0.4 | 4.0 | 1.1 |
| Diameter of lumen | 0.4 | 4.0 | 1.2 |

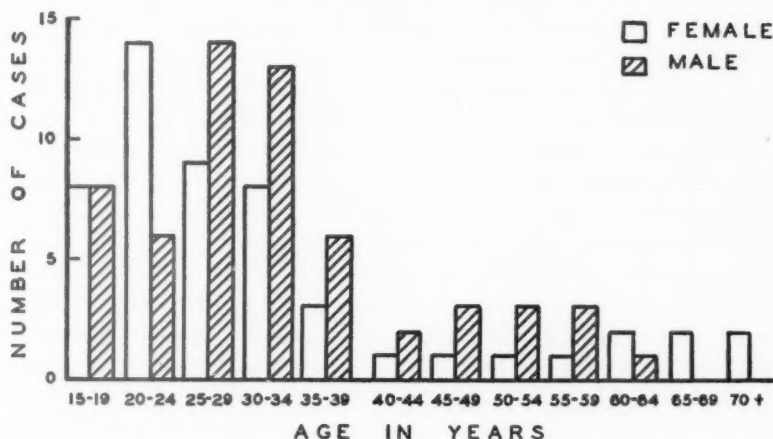
GENERAL FEATURES

Cicatrizing enteritis typically involves a single short segment of terminal ileum, and hence the term regional ileitis is justifiably popular. It is of interest that the jejunum^{28,29} and the cecum or ascending colon³⁰⁻³⁴ also are each affected in about 10 per cent of the cases. It is claimed that the disease is farthest advanced at its distal end and characteristically spreads upward,³⁵⁻³⁷ but instances of distal extension also are reported.^{31,38} The appendix may take part in the inflammatory reaction.^{30,35,39} Occasionally only the jejunum or colon is affected.⁴⁰⁻⁴⁵ The latter condition is rare, and many of the cases⁴⁶⁻⁴⁹ are open to question. Discontinuous "skip areas" of involvement in either or both the large and small intestine^{50,51} also have been described. In 4 reported instances,⁵²⁻⁵⁵ cicatrizing enteritis spread to a Meckel's diverticulum. There are no convincing reports implicating the esophagus, stomach, or duodenum.⁵⁶⁻⁵⁸ The localization and extent of cicatrizing enteritis in the present series will be found in Table I.*

*During the same period there were 240 cases of ulcerative colitis.

Most of the patients are less than 40 years old, and men and women are affected equally (Text-Fig. 1). Occasional familial cases are reported.^{59,60} There is no good evidence of special racial susceptibility. The names of patients in the present series indicate origins from all of the major stocks of the nation without any unusual predilection being apparent. One case in a Negro is included.

Cicatrizing enteritis usually is observed by the pathologist in the chronic stage, and in the present series of 120 unselected cases, 112, or 93 per cent, were of this type. Reports of more than 99 acute and 22 subacute cases were found in the literature consulted.



Text-Figure 1. Age incidence and distribution by sex of 120 cases of chronic cicatrizing enteritis.

THE ACUTE PHASE

Acute terminal ileitis is rather common, as any surgeon will testify, yet the pathologist rarely has an opportunity to examine material from this condition. Clinically, these cases are indistinguishable from acute appendicitis, but as a rule the appendix is not involved grossly. The terminal 4 to 50 cm. of ileum and its mesentery are thickened, edematous, and hyperemic. There may be a small amount of exudate on the serosa, and at times a little cloudy or clear free fluid in the peritoneal cavity. Characteristically, the segment of inflamed bowel is red-purple or maroon,^{61,62} with a sharp distal margin at the ileocecal valve and often an equally abrupt transition to normal appearing intestine at the proximal boundary. The acute form of the disease is more severe in young children, since the diameter of the bowel is smaller and edema easily produces obstruction.⁶³⁻⁶⁵

Information concerning clinical sequelae of acute ileitis is meager, but among 15 acceptable cases^{66,67} 12 patients (80 per cent) regressed, 2 (13 per cent) progressed, and one (7 per cent) died. Because of the general impression that most patients with acute ileitis recover spontaneously, the surgeon who finds this condition at operation usually either removes no tissue or performs an appendectomy.⁶⁸⁻⁷² The appendices in 30 such cases^{37,39,73-78} were described as follows: acutely or subacutely inflamed in 2 cases each, chronic periappendicitis in 10, fibrosis in 3, lymphoid hyperplasia in 15, and negative in 8 cases. Two appendiceal mucoceles were present.^{61,79} Mesenteric nodes examined in 7 cases were acutely inflamed in 4 and hyperplastic in 5 cases. A history of previous appendectomy is given in 28 per cent⁶⁸ to 50 per cent¹⁶ of all cases of cicatrizing enteritis.

Dalziel⁶ found the earliest histologic changes to be congestion, submucosal edema, and irregular hemorrhages in the intestinal mucosa and submucosa. Crohn *et al.*¹⁶ described as the primary lesions oval mucosal ulcerations about 1 cm. in diameter with axial polarity, located especially beneath the attachment of the mesentery. Ectasia of lymph and blood vessels and infiltration of polymorphonuclear leukocytes were present throughout all layers.

Erb and Farmer⁷³ reported the best documented case of acute ileitis and also the youngest: a 2½-year-old girl who died on the eighth day of illness. Salient autopsy findings included peritoneal effusion, marked edema of the terminal ileum, cecum and proximal colon, with closure of the ileocecal valve, membrane-covered ulcers on the mucosal surfaces of Peyer's patches, and enlargement of the regional lymph nodes. Microscopically, edema was most prominent in the submucosa but involved the subserosa also. The inflammatory exudate consisted mostly of large endothelial leukocytes with pale vesicular nuclei. There were also some polymorphonuclear cells and striking necrosis of lymph follicles. Some small veins were thrombosed and lymphatics were distended with granular pink material. Sinusoids of the lymph nodes were widely dilated and at times filled with large mononuclear cells.

In our series there was acute inflammation in 8 cases, but 5 of these represented acute exacerbations of cicatrizing enteritis of longer duration. The 3 regarded as true acute cases occurred in women of 22, 25, and 36 years of age. The gross and microscopic changes were similar to those described by Erb and Farmer.⁷³ The acute inflammatory exudate and edema were accompanied by numerous large, round monocyctic cells identical with Mallory's endothelial leukocytes,⁸⁰ lying free or clumped in lymphatic spaces and in interstitial tissues. The blood

vessels and their endothelial cells took no active part in these processes. Ulceration was present in 2 cases, and in one the acute reaction extended into the colon from the ileum.

THE SUBACUTE PHASE

Because cicatrizing enteritis is characterized by remissions and exacerbations usually of indefinite duration, the subacute stage is not sharply distinguishable from the acute or chronic forms.^{81,82} Histories usually describe abdominal pain and indigestion of 2 to 6 months' duration.^{83, 84} Of the 6 reported cases of cicatrizing enteritis in which trauma was considered a causative factor, 4 had preoperative histories of difficulty for 2 to 4 months, and 5 showed gross and microscopic subacute cicatrizing enteritis.⁸⁵⁻⁸⁸ The ileocecal junction frequently is severely affected in the subacute and chronic stages and may be stenosed as a result of inflammation.^{16, 20, 25-27} The changes in the bowel, mesentery, and regional lymph nodes are more extensive and intense than those seen in the acute phase and consist of a mingling of acute and chronic characteristics. Grossly, the intestine has some of the hyperemia of the acute phase, and also the boggy generalized edematous thickening of chronic enteritis. The bowel may be very friable as a result of extensive ulceration extending through the submucosa.^{89,90} The mucosa often has a reticulated pattern produced by coalescence of ulcers, leaving scattered islands of swollen mucosa. The ulcers are covered by a fibrinous or diphtheritic membrane and the inflammatory exudate is a mixture of polymorphonuclear leukocytes, lymphocytes, and plasma cells. Eosinophilic leukocytes may be more or less numerous, but they do not characterize the subacute reaction.^{86, 43, 84} The proliferation of endothelial cells of the lymphatics becomes more prominent in the subacute stage and giant cell granulomas begin to appear. These will be described at length later. In the mesentery and lymph nodes there are similar but milder inflammatory reactions.

Our group includes 5 subacute cases. All occurred in women, aged 20, 20, 23, 30, and 56 years. One involved only 1.5 cm. of sigmoid colon.

THE CHRONIC PHASE

A large majority of all cases of cicatrizing enteritis have a previous history of months or years of intermittent diarrhea and cramping abdominal pain, with intestinal stenosis. Intestinal obstruction may be observed⁹¹⁻⁹⁴ but perforation with peritonitis is unusual.^{18, 95, 96}

The gross appearance is often characteristic enough to be diagnostic (Fig. 1). A sharply demarcated segment of intestine is indurated,

soggy, leathery, and is often compared to a rubber garden hose in size and consistency.^{5,97-101} The wall is commonly thickened to about 1 cm. with participation of all layers, and the lumen may be irregularly narrowed to 0.5 cm. or less (Fig. 2). At times the distal end of the process will barely admit a probe. Ulceration is not inevitably present, but as a rule there are shallow ulcers in the long axis of the bowel, especially beneath the mesenteric attachment. At times the ulcers are girdling or reticulated in pattern. The remaining mucosa may appear atrophic, normal, or hypertrophic and polypoid. The serosa is hyperemic, dull, and occasionally bears white pinhead tubercles.¹⁰²

Mesenteric fat surrounds the intestine to an abnormal extent. The mesentery corresponding to the segment involved is uniformly thickened, stiffened, and contains large edematous lymph nodes. The fibrosed mesenteric leaves produce irregularities in contour of the bowel, which may be slightly corrugated or distinctly shortened in "concertina" fashion.^{103,104} Intussusception is practically unknown, probably because of the restraining influence of the stiff edematous mesentery. Dense fibrous adhesions fixing the diseased bowel to adjacent structures are frequent. Proximal to the diseased portion there is usually both dilatation and hypertrophy of the bowel, occasionally with diverticular outpouchings.

Roentgenologic study of an affected ileum may show defective filling, an irregular or tapering intestinal pattern proximal to the segment most severely involved, or occasionally the characteristic "string sign" produced by a thin stream of barium passing through the stenotic lumen.¹⁰⁵

Relatively few autopsy reports of cicatrizing enteritis are extant,^{58,106-111} but data are sufficient to indicate that characteristic changes have not been found except in the involved intestine and its mesentery and regional lymph nodes. Two of our 120 cases were studied at autopsy. A boy, 19 years old, had had a 3-year history of abdominal distress, and the anatomic findings were cicatrizing enteritis of jejunum and ileum, jejunal sinus, serosal fibrous adhesions of the small intestine with angulation and partial obstruction of the jejunum, healed appendectomy, healed laparotomy incision, serous atrophy of fat, emaciation, and hyperplasia of mesenteric lymph nodes. Death was due to intestinal obstruction. The other necropsy was of a Negro woman, 37 years old, who had regional ileitis, fecal fistula, partial intestinal obstruction, ileo-ileal and ileosigmoid fistulas, a recent ileocolostomy, abdominal and inguinal subcutaneous fecal sinus tracts, ulceration and necrosis of the abdominal skin, thrombosis of the inferior vena cava and exter-

nal iliac veins, echinococcus cyst of the spleen, pigmented lesions of the skin of the face, and an exostosis of the medial epicondyle of the right knee joint. The immediate cause of death was undetermined.

The pathologic histology has usually been described as diffuse chronic inflammation and edema ending in marked fibrosis.^{16, 20, 108, 110-116} There are shallow, beveled, mucosal ulcerations coated with fibrin and inflammatory cells, including polymorphonuclear leukocytes, and beneath them the submucosa is edematous and crowded with plasma cells, lymphocytes, and eosinophils. Exudate of this type infiltrates all of the deeper layers and extends into the mesentery locally. The submucosal and subserosal lymphatics are dilated. The muscle is hypertrophied. The submucous and myenteric nerve plexuses appear prominent because the ganglion cells are swollen and the periganglionic lymph spaces are dilated.*

Focal granulomas frequently are observed scattered through the intestinal wall, in lymph nodes, or in both.^{16, 20, 100, 108, 112, 118-120} These are formed by large mononuclear phagocytes with one or more central giant cells, which are often vacuolated but usually do not contain foreign bodies. While these foci somewhat resemble those of tuberculosis, they are easily distinguishable in most cases, and acid-fast bacilli are not present. The origin and significance of the giant cells are the source of considerable disagreement. Some authors^{9, 16, 26, 118, 121-125} believe that they are foreign body giant cells developing in response to vegetable fibers, crystals and lipids of dietary origin, or parasites,¹²⁶ all of which have been carried by lymphatics from the depths of ulcers. Others^{102, 112, 118, 117, 127-130} consider these giant cells to be identical with the Langhans type.

Hadfield^{102, 127} has presented the most thorough study of the giant cells. In 13 of 20 cases of cicatrizing enteritis, he found obstructive lymphedema and lymphoid hyperplasia in the intestinal submucosa. Pale endothelial cell masses developed in the germinal centers of the lymphoid nodules and eventually replaced them completely. Giant cells then appeared in the centers of these enlarging granulomas, which he termed "giant cell systems." After reaching a certain size, the granulomas thereafter slowly regressed without showing necrosis or fibrosis. Hadfield also found "giant cell systems" in lymph nodes, even when they were not present in the intestine. This fact has not been recognized generally.^{58, 128, 131-133}

* No support has been found for a recent theory that this is the primary lesion, and cicatrizing enteritis a neuropathy.¹¹⁷ In fact, similar changes are found in ganglia of cases with intestinal neoplasms or ulcerative colitis.

In the present series there were several chronic cases which were in a stage sufficiently early to permit better evaluation of the processes involved than was possible with the few acute and subacute examples available for study. The degree of ulceration and secondary enteric infection is quite variable; often it blots out any previous pathologic change, but at times it is absent. Examination of appropriate specimens has shown the existence of sequential changes, which have not been described previously, leading to the obstruction of lymphatics and formation of granulomas in the intestinal wall and lymph nodes.

In the earliest stage, small foci of leukocytes closely resembling the endothelial cells of lymphatics appear in the lacteals of the lamina propria, between the glands and the muscularis mucosae. These endothelial cells change from flat to polygonal, with abundant granular eosinophil cytoplasm and somewhat prominent hyperchromatic nuclei. Proliferation of these cells continues and finally blocks the lymphatics (Fig. 3). In slightly later stages similar masses of proliferating endothelium obstruct lymphatics in the submucosa and subserosa (Fig. 4). Mitotic figures are found among these cells. The reaction is sharply focal and intervening stretches of the lymphatic are dilated. There is accompanying edema, but no local necrosis or inflammatory exudation; in fact the formation of granulomas is best observed in places devoid of inflammatory cellular infiltration.

Once the larger lymphatics become completely blocked deep in the submucosa and subserosa as well as in the lamina propria, eosinophils and then lymphoid cells surround these endothelial masses in increasing numbers. The endothelial cells become more closely massed and tend to coalesce, forming giant cells. Incomplete stages in this process can be found in which individual endothelial cells are partly fused. The giant cells often contain vacuoles or polymorphonuclear leukocytes, but only rare giant cells in 11 of 61 cases contained ingested foreign bodies. The vacuoles are frequently marginal and give the giant cell a scalloped appearance, making it difficult to determine whether these are fluid inclusions which do not stain for fat or cytoplasmic threads clinging to the edges of the original lymphatic and producing a false appearance of vacuolization (Fig. 5). In some cases the granulomas resemble atypical lymph follicles (Fig. 6).

Of the 120 cases, 100 (83 per cent) showed significant granulomatous response in the intestine. This was marked in 68 cases, and in 32 it required search amid the secondary inflammation to find acceptable foci. Giant cells were present in 51 specimens of intestine (43

per cent). Lymph nodes were studied in 93 cases, and 67 (72 per cent) showed endothelial response of the type described. The response was marked in 54 of these cases, and 28 (30 per cent) contained giant cells. There was no formation of granulomas at all in 12 (10 per cent) of the 120 cases, but in 4 of these no lymph nodes were isolated for study.

Necrosis within these granulomas is uncommon whatever their location, but was present in 8 of 61 cases (13 per cent). The centers are replaced by coarsely beaded red material of so-called fibrinoid appearance, and in 3 cases polymorphonuclear leukocytes also were present (Fig. 7). No caseous necrosis was observed. More commonly, these granulomas are replaced slowly by hyaline collagenous material containing scattered shrunken nuclei, and this process sometimes forms a prominent feature histologically.

An identical sequence of changes is found in the mesenteric lymph channels of the affected segment and in its regional lymph nodes (Fig. 8). The granulomas may become very prominent in these lymph nodes, and are not all of the same stage. In fact at times all variations from early thickening and partial or complete desquamation of the sinusoidal lining cells, through well formed endothelial masses, to masses partly or completely replaced by hyaline material are found in nearby nodes. Three cases of the present series had calcified mesenteric lymph nodes, and in one necrosis suggested coincidental tuberculosis. A single granuloma with giant cell was observed in the liver of one of the 2 cases autopsied, and material from a third autopsy which was studied had several similar hepatic granulomas (Fig. 9).

The outstanding secondary process which follows and often obscures the changes described is ulceration. This is not observed until edema of the intestine is well established, and at no stage do the intestinal blood vessels or the lymphoid tissue show any definite microscopic abnormalities which might predispose to the formation of ulcers. As soon as ulcers develop they are surrounded by a mixed exudate of eosinophils, lymphocytes, plasma cells, and polymorphonuclear leukocytes with a variable and usually small amount of fibrin in the ulcer bases. The ulcers extend to, or sometimes into, the muscularis, surrounded by heavy collars of granulation tissue. Nonspecific acute and chronic inflammation now merges with the distinctive primary granulomatous process and it can no longer be distinguished.

Formation of fistulas by extension of this ulceration to abdominal or perianal skin often attracts clinical attention and leads to the diagnosis

of cicatrizing enteritis.¹³⁴⁻¹⁴⁰ In different series fistulas were reported in 23 to 36 per cent of the cases. In Table II is shown the incidence in our series. It is estimated that at least 10 per cent of all fecal fistulas are caused by cicatrizing enteritis.¹³⁸ Pathologic study of the tracts has revealed no special characteristics.¹³⁴ Abscesses often are formed as a by-product of the fistulas. The rarity with which peritonitis is encountered in cicatrizing enteritis is due to the slow extension of secondary inflammation, which may be retarded by the widespread lymphatic obstruction already present.

TABLE II
Complications of Cicatrizing Enteritis

| | Number of cases | Percentage |
|-----------------------------------|-----------------|------------|
| Adhesions | 31 | 26 |
| Total fistulas: | 32 | 27 |
| Intestine-intestine | 12 | 10 |
| Intestine-skin | 12 | 10 |
| Into mesentery or retroperitoneum | 7 | 6 |
| Sigmoid-bladder | 1 | .. |

Reparative tendencies are found in chronic cases, with fibrosis of the inflamed wall, and the ulcers are covered by a thin layer of primitive or metaplastic epithelium.³⁰ The muscular layers are hypertrophied. One case of mucinous adenocarcinoma of the ascending colon, found apparently arising in intestine affected by cicatrizing enteritis, is included in the present series. The relationship may be coincidental, as there are no similar cases in the literature.

Resection is the surgical treatment of choice, and this is said to cure 83 to 85 per cent of the cases.¹⁴¹⁻¹⁴³ On the other hand, simple side-tracking operations¹⁴⁴ are successful in from 49 to 87 per cent. Attention has been drawn in the literature to postoperative recurrences of cicatrizing enteritis proximal to the resected segment in a total of 11 cases. Recurrence may become apparent clinically as late as 10 years after operation.^{145, 146} One woman who died of unrelated cause 9 years after an ileocolostomy for regional ileitis had at autopsy a terminal ileum of which the lumen had been almost completely occluded by a contracted indurated mesenteric sheath.¹⁰⁹ Microscopically, this unused segment of ileum still showed chronic inflammation of the mucosal surface, edema and thickening of the wall, with muscular hypertrophy.

Medical treatment also has its adherents.^{147, 148}

DIFFERENTIAL DIAGNOSIS

The problem of distinguishing between acute cicatrizing enteritis and dysentery,¹⁴⁹⁻¹⁵⁴ typhoid fever,^{155, 156} amebic dysentery, "intestinal flu,"¹⁵⁷ or the effects of chemical irritants^{158, 159} does not arise very often. When it does, all available clinical and laboratory data usually are required to make the proper diagnosis.

Chronic cicatrizing enteritis, in its characteristic form, is not easily confused with other lesions. Differentiation from tuberculosis is probably the most troublesome.¹⁶⁰ Intestinal tuberculosis is usually widespread, and the organisms are present in the stool. Clinically well studied cases of cicatrizing enteritis have shown no evidence of tuberculosis.¹⁰² Microscopically, the presence of granulomas with giant cells provides the main source of difficulty in distinguishing the two diseases. The granulomas of cicatrizing enteritis have a disorderly appearance with indistinct cell boundaries, the endothelial cells are mixed with lymphocytes, and the giant cells are scalloped and irregular in size and shape. Caseous necrosis is lacking and acid-fast bacilli are absent. The term cicatrizing enteritis has now absorbed almost all of the cases once called hyperplastic tuberculosis of the intestine, and the latter term is practically obsolete.^{161, 162}

The resemblance between the granulomas of cicatrizing enteritis and sarcoid has been noted by many authors, some of whom have considered them morphologically indistinguishable.^{87, 102, 121, 126, 127, 130-132, 163} Two recent cases¹³⁰ reported as "isolated sarcoidosis of intestine" showed noncaseous tubercles in the small intestine, negative tuberculin tests, and negative stool studies for tubercle bacilli. The evidence presented, however, did not convincingly exclude them as examples of cicatrizing enteritis. Blackburn *et al.*¹⁰² failed to find positive evidence of sarcoidosis in 16 cases of regional ileitis. Analysis of 50 autopsied cases of sarcoid¹⁶⁴⁻¹⁸⁰ is of interest in this connection. Seven of these cases had some involvement of the intestine, which was tuberculous in 2,^{164, 176} sarcoid generalized through stomach and bowel in 3,^{169, 174, 177} local sarcoid of the descending colon in one,¹⁶⁶ and an indeterminate granuloma possibly complicated by syphilis in one case.¹⁶⁸ Microscopically, the granulomas of sarcoid are larger, more numerous, and lack the lymphocytic infiltrate seen in cicatrizing enteritis. The spiculated bodies seen in the giant cells of sarcoid were absent from all of the giant cells of the 61 appropriate cases of cicatrizing enteritis in our series. Therefore at present one must reject the idea that sarcoidosis and cicatrizing enteritis are the same disease.

While the granulomas of cicatrizing enteritis are essentially different in structure from those of sarcoid and tuberculosis, they are not pathognomonic. Identical cellular aggregates sometimes are observed in diverticulitis, cancer, and lymphoma of the intestine. At least 3 otherwise typical cases of ulcerative colitis, which we have observed, contained these granulomas in the colon and regional lymph nodes.

Ulcerative colitis involves the ileum in about 25 per cent of all cases, and clinically may be difficult to distinguish from cicatrizing enteritis.^{44, 68, 181} However, the pathologist usually finds distinct differences. No matter how extensive ulcerative colitis becomes, the intestine does not undergo the marked fibrotic and edematous thickening found in cicatrizing enteritis. Microscopically, in ulcerative colitis one finds acute inflammation with fibrin, hypersecretion of mucus, edema, necrosis, sloughing of the mucosa, intense inflammation, granulation tissue, and fibrosis all taking place chiefly in the mucosa and submucosa.^{182, 183} The sluggish, progressive inflammation with tubercle-like structures of cicatrizing enteritis involves all layers of the intestine and is accompanied by more marked edema, fibrosis, and muscular hypertrophy.

Regional colitis,^{161, 184, 185} which attacks the proximal colon and spares the sigmoid, and hyperplastic colitis,¹⁸⁶ in which a localized inflammatory mass is formed, apparently do not differ pathologically from ulcerative colitis. One must, however, be prepared to recognize skip areas of cicatrizing enteritis in the proximal colon.

Amebic dysentery reaches the terminal ileum only in a late generalized stage. In the older cases fibrosis may cause considerable thickening of the bowel, but this is seldom comparable to the amount found in cicatrizing enteritis, and the distribution of the ulcerations is a useful distinguishing feature.

Bacillary dysentery affects the colon much more often than the small intestine, producing necrosis of lymphoid tissue leading to ulceration.¹⁸⁴ Chronic edematous induration of intestine and mesentery is not found as in cicatrizing enteritis. Microscopically, the inflammation in dysentery is located mainly near the mucosal surface, and there is no diffuse granulomatous process infiltrating the intestine, mesentery, and regional lymph nodes.

Lymphogranuloma venereum of the colon occasionally may suggest cicatrizing enteritis grossly.¹⁸⁷ Granulomatous inflammation is present, with tubercles the centers of which are necrotic and contain polymorphonuclear leukocytes. Elsewhere there is diffuse plasma cell

infiltration. These microscopic findings do not resemble cicatrizing enteritis. The Frei test will aid clinical differentiation.¹⁸⁸

Talc granuloma, usually caused by excess powder on gloves at laparotomy, may produce a gross appearance indistinguishable from regional ileitis, and the presence microscopically of doubly refractile crystals surrounded by epithelioid or giant cells should always suggest inquiry as to this possibility. Lichtman and co-workers^{189,190} found crystals in about 25 per cent of 198 cases at the Mayo Clinic, originally called noncaseous tuberculosis of ileum, and believed that 33 (16 per cent) contained enough doubly refractile crystals to be of etiologic significance. They listed more than thirty-five other pathogenic agents which could produce pseudotuberculous granulomas. Five of our cases contained doubly refractile material, but only one had extensive deposits. The specimen was from the jejunum of an 18-year-old girl who had no previous operation, and the origin and significance of the crystals are unknown.

ETIOLOGY

Most clinical authors have considered cicatrizing enteritis to be an infection of unknown cause,^{97,102,114,191} and bacteriologic studies of many cases have failed to reveal a specific agent. In a case of Erb and Farmer⁷³ a nonmotile *Escherichia coli* variant was isolated in pure culture from the intestine, blood, and abdominal organs. Three of their 6 patients with acute ileitis possessed serum agglutinins against this same organism in a titer higher than 1:160. Attacks of acute ileitis also have been associated with severe pharyngitis¹⁹² or diphtheria.¹⁹³ Various bacteria of doubtful pathogenicity, such as anerobic streptococci,¹⁹⁴ *Bacillus proteus*, and *Aerobacter aerogenes*^{74,195} also have been isolated. Pumphrey¹⁹⁶ commonly found no growth of bacteria from uncontaminated surgical specimens. No positive evidence of viral etiology has been reported.

Numerous other suggestions have been made. Felsen¹⁵⁰⁻¹⁵⁴ considered both cicatrizing enteritis and ulcerative colitis to be related to bacillary dysentery. Trauma and lack of good collateral circulation have been blamed.⁸⁵ Sympathetic hyperexcitability of the terminal ileum has been suggested.¹⁹⁷ Some writers have implicated allergy, partly because of the numerous eosinophils present.^{81,198} Other articles^{30,135} have stated that the disease is a complication of appendicitis, but most authors agree with Mixer¹⁹⁴ that an unoffending appendix frequently is removed. The primary site of cicatrizing enteritis is generally thought to be in the intestine,^{21,32,50,118,199} but a few writers have

concluded that disease of mesenteric lymph nodes is the cause.^{39, 191, 198, 200}

None of these ideas is entirely acceptable, and the etiology remains unknown. Pugh¹⁰⁴ remarked that absence of a known etiologic agent is a prerequisite for the diagnosis of cicatrizing enteritis. The microscopic picture is that of a response to lipid, such as is produced experimentally by injection of animal oils, according to Pinkerton.¹³¹ Fat stains of the granulomas and giant cells in 5 cases of the present series were weakly positive. The granularity of the cytoplasm of the giant cells suggested finely divided lipid. It is suggested that fat-containing chyle may escape from the damaged lymphatics and act as a chronic irritant to interstitial tissues.

PATHOGENESIS

That cicatrizing enteritis is a clinical, but not a pathologic entity^{22, 97, 111, 131, 201} has been the general opinion held by most physicians, despite claims to the contrary.^{97, 127} It is true that in the late ulcerated and fibrotic stage with its microscopic picture of banal chronic inflammation, fibrosis, abscesses, and fistulae, characteristic features rarely will be found. But observation of earlier lesions has, we believe, shown a sufficiently uniform process of special type to warrant considering cicatrizing enteritis a pathologic entity. Sections taken through the most severely ulcerated and secondarily infected portions of intestine are less characteristic microscopically than those from less damaged intestine, particularly around the proximal edge of the grossly changed segment. Likewise, the largest available lymph nodes more often show only inflammation and edema, while medium-sized nodes are richer in granulomatous endothelial and giant cell foci.

Lymphatic obstruction has been favored by many authors^{117, 148, 199, 202, 208} as the most important factor in the development of cicatrizing enteritis, but has been observed previously only by Blackburn *et al.*¹⁰² According to these authors, the primary lesion is a specific hyperplasia of lymphadenoid tissue in the submucosa and the regional lymph nodes. The lymphatics are obstructed by lymphocytes. In our series lymphatic blockade also was observed, but was the result of endothelial cell proliferation and desquamation. Superficial erosions may be induced by the marked edema and in time be followed by secondary infection, lumpy abscesses, sinus tracts, fibrosis, and adhesions. Strömbeck⁶⁰ explained the predilection of the ulcers for the mesenteric side of the bowel on the basis of fixation of the vessels by phlegmonous edema of the mesentery.

The experimental work of Reichert and Mathes²⁰⁴ adds further

evidence in support of the fundamental significance of lymphatic block. Sclerosing solutions injected into cannulated lymphatics in the intestines of dogs produced gross and microscopic changes resembling cicatrizing enteritis. Almost all of the animals showed acute lymphedema, and the lymphatics were filled and blocked by large pale cells. After 1 month, chronic lymphedema with greater submucosal thickening was observed, but the inflammatory cells disappeared. Intravenous introduction of *Escherichia coli* enhanced these changes. Sclerosis of the intramural intestinal lymphatics was not achieved; had it been, more severe mucosal changes probably would have occurred. The conclusion drawn was that both low-grade chronic infection and chronic permanent lymphedema formed the basis of regional cicatrizing enteritis.

The suggestion^{10, 104, 110, 205} that the primary lesion is a proliferation of interstitial elements which may interfere with the blood supply and thus cause necrosis, ulceration, and cicatrization does not explain the characteristic edema. Again the hypothesis of minor superficial ulcers as a portal of entry for a slowly spreading infection of low virulence^{32, 206} is open to objection, since numerous cases show marked disease of the bowel with slight or absent ulceration.

SUMMARY

One hundred and twenty unselected cases of cicatrizing enteritis have been analyzed together with a comprehensive review of the literature on the acute, subacute, and chronic stages of the disease. The terminal ileum of a young adult is most often affected, in either sex and of any race, but the jejunum, upper ileum, appendix, cecum, or colon may be involved. The etiology is unknown. The characteristic gross findings are sharply demarcated induration and edema of intestine and its mesentery, with enlargement of regional lymph nodes. Microscopic sequences indicate that swelling and proliferation of lymphatic endothelium in intestine and lymph nodes cause occlusion of lymphatics and resulting edema. Granulomas containing giant cells are formed by these cells throughout the intestinal wall and in mesentery, lymph nodes, and liver. These granulomas slowly hyalinize, usually without necrosis, or are obscured by secondary bacterial infection. In late stages, subacute and chronic inflammation, fibrosis and muscular hypertrophy of the intestine are prominent. Cicatrizing enteritis is an acceptable pathologic entity.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 88

- FIG. 1. Cicatrizing enteritis of the terminal ileum, sparing the cecum and appendix. Thickening of the small intestine and its mesentery is present, with stenosis of the lumen. The patient was a boy, 16 years old.
- FIG. 2. Ileum from cicatrizing enteritis on the left. Normal ileum is on the right.
- FIG. 3. Ileum of early chronic cicatrizing enteritis, showing granulomas in the lamina propria on each side of the base of a villus. Phosphotungstic acid-hematoxylin stain. $\times 40$.



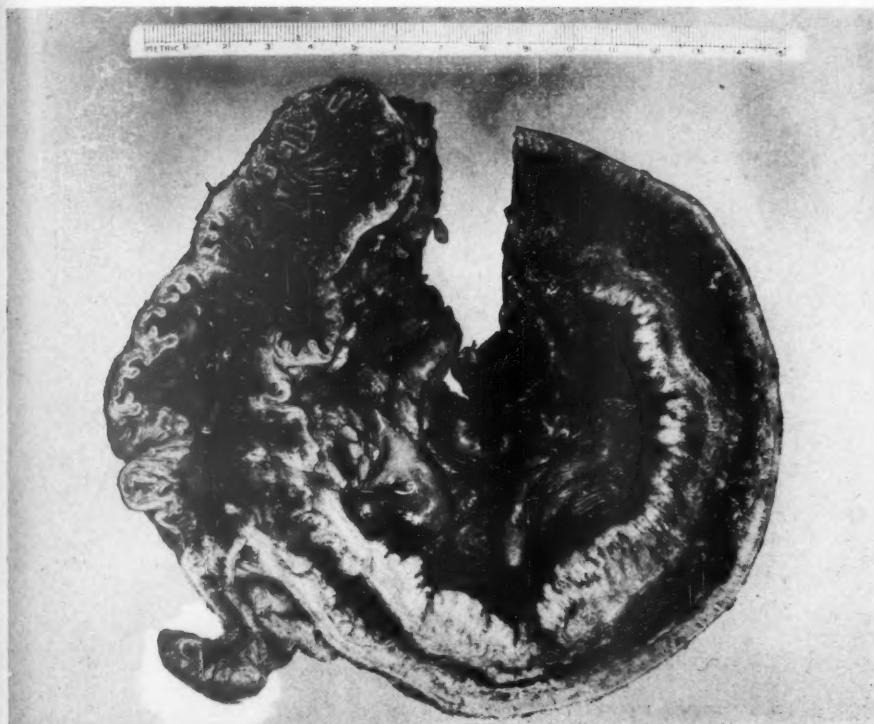
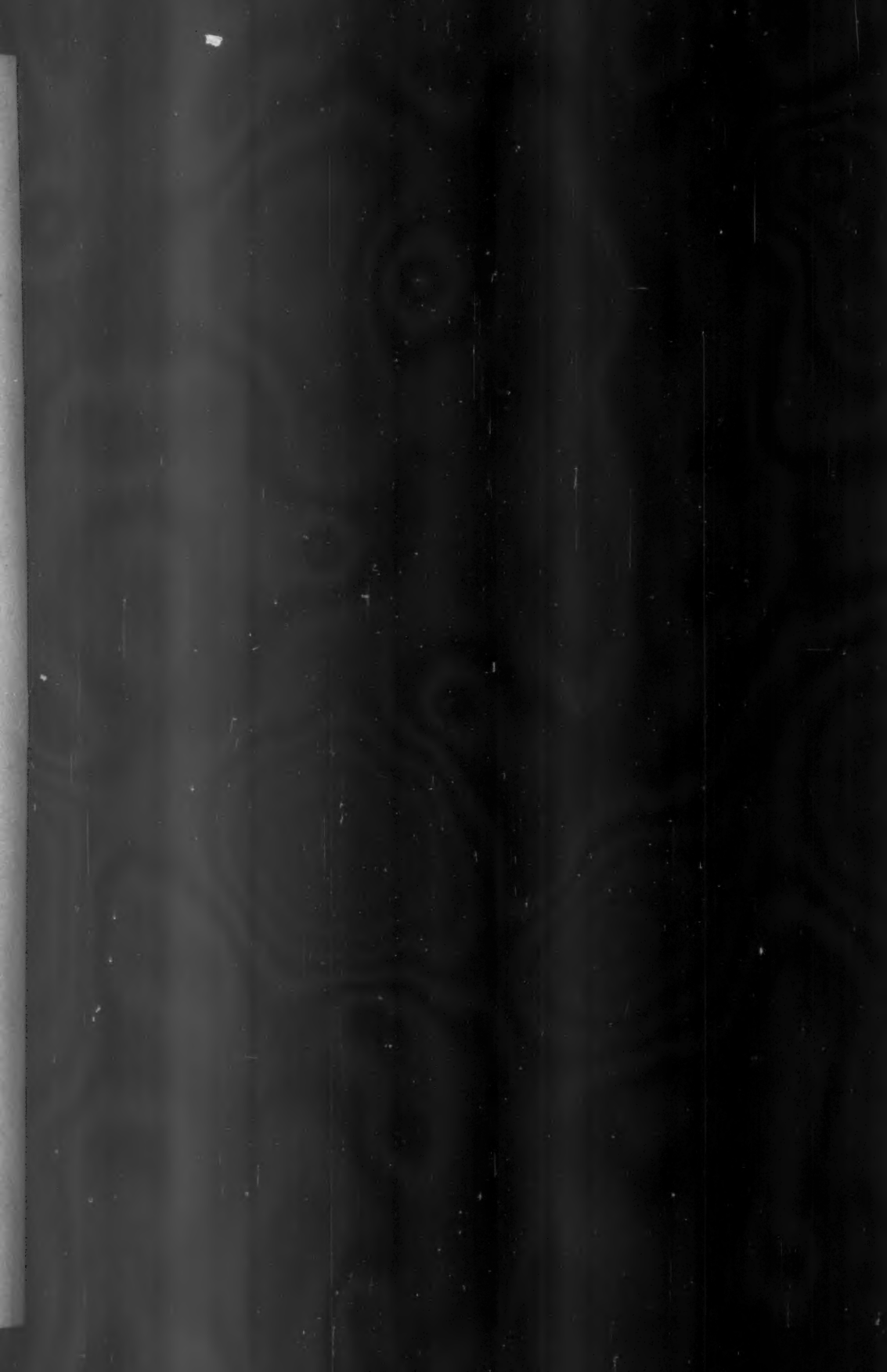
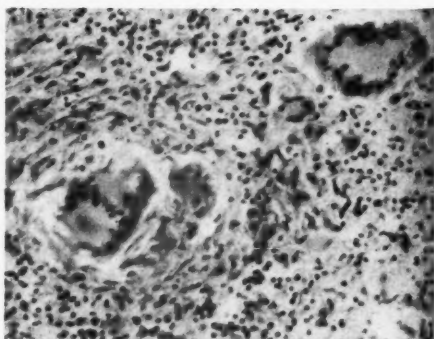


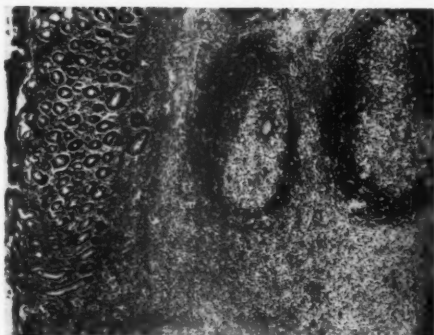
PLATE 89

- FIG. 4. Ileum in chronic cicatrizing enteritis, showing endothelial cells proliferating and blocking a submucosal lymphatic. Phosphotungstic acid-hematoxylin stain. $\times 800$.
- FIG. 5. Lymph node. Giant cells in a granuloma, showing different stages of formation, and peripheral scalloping. Phosphotungstic acid-hematoxylin stain. $\times 200$.
- FIG. 6. Fully developed granulomas in the submucosa beneath intact mucosa. These simulate lymph nodules. Phosphotungstic acid-hematoxylin stain. $\times 30$.
- FIG. 7. Lymph node. Necrosis in a granuloma of cicatrizing enteritis. The intestinal granulomas in this case also showed necrosis. Phosphotungstic acid-hematoxylin stain. $\times 125$.
- FIG. 8. Granuloma around a lymphatic in the mesentery of cicatrizing enteritis. Phosphotungstic acid-hematoxylin stain. $\times 200$.
- FIG. 9. Granuloma in the liver of an autopsied case of cicatrizing enteritis. Phosphotungstic acid-hematoxylin stain. $\times 200$.

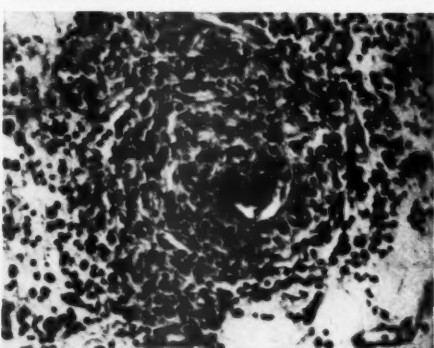
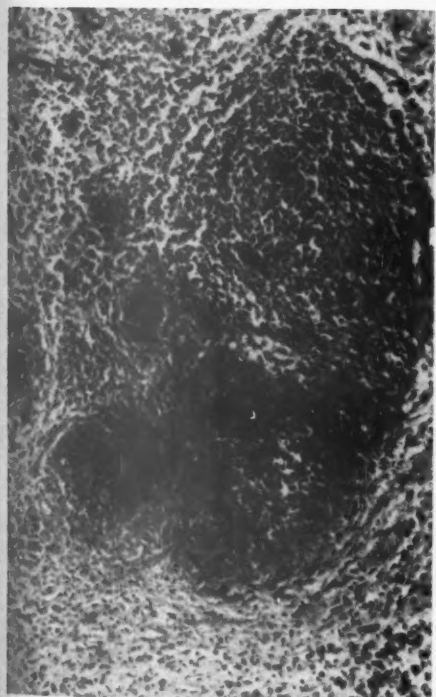




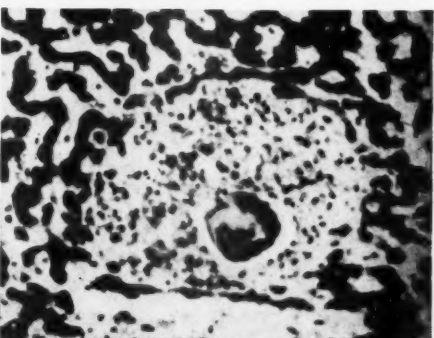
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HEPATIC AND RENAL INJURY WITH CALCIUM DEPOSITS AND CIRRHOSIS PRODUCED IN RATS BY PYRIDINE*

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Observations made in a series of nutritional and chemical investigations of the mechanisms of the hepatic and renal injury produced by pyridine are presented in this report. Pyridine was chosen for use in these studies because of the possibility that it might, by its methylation in the body,¹ cause hepatic and renal injury by draining the labile methyl groups from choline and methionine, and thus produce an "intrinsic" deficiency of these substances.^{2,3} The observations that pyridine produced hepatic and renal injury, which was prevented to a considerable extent by methionine,² and that the already methylated product of pyridine, in equivalent amounts, did not produce the lesions,⁴ seemed to be in favor of this hypothesis. However, because of the failure to obtain the methylated product from the urine of the animals fed pyridine, while it was readily obtained from those fed the already methylated product itself,⁴ and particularly because of the ineffectiveness of methyl-containing choline in preventing death of the animals, but effectiveness of non-methyl-containing cystine (although the efficacy of cystine was markedly enhanced by simultaneous administration of choline),⁵ it now appears that if this mechanism played a part in the observed toxicity of pyridine, it was not the only or most important one.⁵ Possible mechanisms of action of pyridine in causing renal and hepatic damage will be considered in other reports.

Pyridine is included in the structure of many biologically active substances, and is formed in small amounts in the burning of tobacco and the roasting of coffee. Its toxic actions, particularly on protracted administration, have not been studied extensively, and apparently there have been no investigations of the effects of its administration in the diet. Pyridine has not been regarded as a highly toxic substance by most investigators. Pollock, Finkelman, and Arieff,⁶ in 1943, reviewed the subject of pyridine toxicity and reported the effects of prolonged administration to 5 patients, 2 of whom became seriously ill,

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apparently due to hepatic and renal damage. Pyridine usually has not been listed among the substances producing hepatic and renal injury,⁷ however, and there have been no reports of the production of cirrhosis by means of this agent.

EXPERIMENTAL MATERIALS AND METHODS

Animals. More than 300 rats have been fed diets containing pyridine, with or without other supplements, for periods up to 4 months. At the end of 4 months on the pyridine-containing diets, some of the animals were placed on stock diet and have now been observed for an additional 2 months, although few of the latter animals have been autopsied.

In most experiments, young male rats (Sprague-Dawley or Sherman) after weaning were placed for about 1 week on stock diet and then transferred to the experimental diets, some of which contained pyridine. Control groups were run in each experiment. Older rats have been used to a limited extent, with results which did not differ markedly from those obtained with young rats.

Pyridine. In the early experiments, redistilled pyridine was added to the diets and considerable care exercised to prevent loss by evaporation. In later experiments, pyridine citrate (A. D. Mackay Co.) was used, and the effects apparently were in no way different from those of equivalent amounts of pyridine itself. Pyridine also was administered by injection to small groups of animals. The effects of pyridine given daily by subcutaneous injection, and by stomach tube during a fasting period, were compared, in paired-feeding experiments, with the effects of equal amounts of pyridine given in the diet. Pyridine itself, rather than pyridine citrate, was used for the injections, because of the severe irritating effect of the latter agent.

Diets. In the beginning, because of the idea expressed above concerning the mechanism of action of pyridine, it seemed desirable to use an experimental diet low in choline and methionine, but not so low that pathologic lesions would result from the diet alone, during the experimental period. Later, this diet seemed undesirable because in spite of control groups, it was difficult to be certain that the inadequate, or at least suboptimal, diet was not partially responsible for the lesions observed. A diet which was higher in protein was then used, and finally a diet containing optimal levels of casein and choline was adopted. The biologic value of this third diet was not increased by further additions of casein and choline.

The compositions of the diets are shown in Table I.

Choline Content of Diets. Diets 1 and 2 contained no added choline. The yeast, starch, and casein per kg. of these diets contained approximately 250 mg. of choline as determined by Glick's modification⁸ of the Reineckate method. On these low-choline diets, young animals which were above the most susceptible age never died with the hemorrhagic-kidney syndrome⁹ of choline deficiency, apparently because of the small food intake and an effect of the starch which has been investigated more fully and reported elsewhere.¹⁰ Animals on diet 1, and to a

TABLE I
Composition of Experimental Diets

| | No. 1 | No. 2 | No. 3 |
|------------------------------------|-------|-------|-------|
| Casein* | 10% | 18% | 25% |
| Lard | 20 | 20 | — |
| Hydrogenated vegetable oil | — | — | 10 |
| Corn oil | — | — | 1 |
| Sucrose | 30 | 22 | 51 |
| Corn starch ("Argo") | 29 | 29 | — |
| Salt mixture (Osborne and Mendel†) | 4 | 4 | 4 |
| Yeast | 5 | 5 | — |
| Cod liver oil | 2 | 2 | — |
| Vitamin supplements and choline | — | — | + |

Supplements per kg. of diet 3

| | | | |
|-------------------|---------|----------------------------------|-----------------------------|
| Thiamine chloride | 20 mg. | Para-amino-benzoic acid | 10.0 mg. |
| Pyridoxine | 20 mg. | Biotin | 0.1 mg. |
| Riboflavin | 30 mg. | Folic acid | 0.2 mg. |
| Ca pantothenate | 50 mg. | 2-Methyl-1,4-naphthoquinone | 1.5 mg. |
| Nicotinic acid | 200 mg. | dl- α -Tocopherol acetate | 15.0 mg. |
| Inositol | 500 mg. | Vitamin A conc. | 60,000 U. S. P. units |
| Ascorbic acid | 500 mg. | Vitamin D conc. | 6,000-10,000 U. S. P. units |
| Choline chloride | | 2.0 or 3.0 gm. | |

* Only the casein used in diet 3 was vitamin free.

† Osborne, T. B., and Mendel, L. B. The relation of growth to the chemical constituents of the diet. *J. Biol. Chem.*, 1913, 15, 311-326.

lesser extent on diet 2, grew more slowly than those on diet 3, but no extensive pathologic lesions were observed during the experimental periods on these suboptimal diets (up to 2 months).

Examination of Urine and Blood. A number of animals were kept in metabolic cages and the urine collected. The specimens were examined for albumin, and in a few instances for blood, sugar, and pigments. Hemoglobin determinations were done colorimetrically on samples of tail blood from a small group of rats, before and after 2 weeks on the pyridine-containing diet. The animals were then sacrificed and the blood examined spectroscopically. Urea and sugar determinations were made on the blood of a few animals in the acute stages of illness due to pyridine.

Examination of Animals and Tissues. Animals were autopsied immediately after death or when death appeared imminent, and the organs examined grossly. Other animals were sacrificed at various intervals in order to study the different stages of injury.

Organs were then fixed in 10 per cent neutral formalin or absolute alcohol. Sections of livers and kidneys of most of the animals and sections of other organs of a few animals were examined microscopically after staining with hematoxylin and eosin. The following stains or methods also were employed in some cases: potassium ferrocyanide-hydrochloric acid for iron, Giemsa's stain, sudan IV, carbol fuchsin, Mallory's phosphotungstic acid hematoxylin, Masson's connective tissue stain, and von Kossa's silver nitrate method for calcium, with and without previous treatment of sections with 5 to 10 per cent concentrated hydrochloric acid. A few unstained sections were examined for pigments and highly refractile substances with visible light, and for fluorescent material with ultraviolet light.

Influence of Diet on Results

The results of feeding pyridine in the different diets were essentially the same, with a few exceptions. Raising the casein level increased somewhat the resistance of the animals to the effects of pyridine, so that it was necessary to increase the pyridine levels simultaneously, in order to obtain approximately the same results. The pyridine levels that have been used with the various diets in most cases were as follows: diet 1, 0.34 per cent pyridine citrate (or 0.1 per cent pyridine); diet 2, 0.7 per cent pyridine citrate; and diet 3, 0.7 to 1.0 per cent pyridine citrate.

The animals placed on diet 1 containing pyridine usually showed a decreased food intake and immediate cessation of growth, whereas those fed diets of higher protein level plus pyridine continued to eat and grow fairly normally even to the time of death. The third difference observed was the presence of more fat and the earlier development of extensive fibrosis in the livers of the pyridine-treated animals on diet 1, and perhaps on 2, than on 3. Thus it was apparently possible, by raising the choline and casein levels, to reduce the fatty changes and fibrosis, without reducing the necrosis.

Rather large vitamin supplements were used with diet 3 to insure adequate intake of vitamins even in cases in which the food intake decreased to low levels. A small number of animals were run on the same diet with yeast and corn starch added, with no appreciable difference in results. Since diet 3 was used in most of the recent studies, and

since the lesions observed on this diet undoubtedly were due entirely to the effects of pyridine, description will be limited to the results on this diet, except when otherwise specified, but it is applicable, for the most part, to the results on the other diets as well.

RESULTS

The animals ate the pyridine-containing diet fairly well and continued to grow, in many cases, at an almost normal rate. In some groups, from 50 to 100 per cent of the animals died in the first week, and in the majority of the groups, most of the animals died within 2 or 3 weeks. Occasionally a considerable number survived for longer periods. Impending death could not always be predicted by the appearance of the animal, but a feeling of coldness as from "shock," which perhaps was in part due to the loss of blood into extensive necrotic areas in the liver, signaled an early end. Survival of the early stages of pyridine treatment apparently resulted in the development of some degree of tolerance.

The principal lesions were found in the livers and the kidneys, and it is presumed that death was due to disturbances of the functions of these organs.

Urine. The urine of the animals frequently became highly colored and contained bile pigment, and sometimes appeared red. Albumin usually was present, sometimes in large amounts, at least during the periods of obvious illness. A few examinations for blood and for sugar were all negative. The urine output sometimes increased markedly in animals which became ill. Anuria, except shortly before death, was not observed.

Blood. Hemoglobin determinations done after 2 weeks on the pyridine-containing diet showed little change from the control values. Characteristic absorption bands of methemoglobin were not noted spectroscopically. Blood chemical determinations revealed normal blood sugars and elevated urea levels.

Serous Cavities. Collections of fluid in the serous cavities were fairly common. Small hemorrhages were noted occasionally beneath the serous membranes. Bile-staining of tissues was seen frequently.

Lungs. An occasional animal exhibited atelectasis of one or more pulmonary lobes. Peribronchial inflammation was noted in some sections.

Lymph Nodes. Mesenteric and peribronchial lymph nodes sometimes appeared enlarged, particularly in animals receiving prolonged treatment.

Spleen. The size of the spleen was quite variable, but in general it seemed to be enlarged in the acute stages of injury, and smaller than normal in the chronic stages. Little hemosiderin was present.

Hepatic and Renal Lesions

Early Hepatic Lesions. The livers of animals dying of acute injury after ingestion of pyridine-containing diets usually were enlarged and darker than is normal, due at least in part to an increased content of blood.

Microscopic sections (Fig. 1) revealed very extensive necrosis with partial dissolution of cells about the central veins and filling of the spaces by red blood cells, most of which seemed to be still within vascular channels. Frequently the only parenchymal cells to escape were those at the periphery of the lobules, particularly in the region of the portal triads, and these sometimes showed degenerative changes.

Animals which survived the initial stage of necrosis usually showed evidence of extensive regenerative activity along with continued injury. Some of the liver cells at the edges of the necrotic areas contained small particles in the cytoplasm which stained dark brown with hematoxylin (Fig. 2). These collections of cytoplasmic bodies were more prominent in the later lesions and will be described in connection with them. Phagocytic cells, particularly in and about the old necrotic areas, contained large amounts of yellow pigment, most of which did not give the Prussian-blue reaction and became green on treatment with acid. Some of the large phagocytes along the central portions of the fibrous trabeculae also contained hemosiderin.

Early Renal Lesions. The kidneys usually appeared swollen, grossly. Sections showed degenerative changes in the epithelial cells, most extensive in the proximal convoluted tubules, but also involving the other segments of the tubules. The cells exhibited granular swelling, hydropic degeneration, some vacuolization, pyknosis of nuclei, and sometimes obvious necrosis. Many pink-staining casts were encountered in the tubules (Fig. 3) and the same material sometimes was present in the glomerular capsules.

Later Hepatic Lesions. More chronic lesions were observed in the animals which survived for longer periods. On gross examination, the livers exhibited a fine nodularity and the cut surfaces showed a distinctly mottled appearance. Greater irregularities of the surfaces, with shrunk and raised areas, sometimes appeared, and in the final periods of study livers were seen with nodules which were considerably larger and lighter in color than those usually seen in cirrhotic livers. The

cut surfaces of these nodules appeared compact, homogeneous, somewhat translucent, and distinctly different from the remaining liver tissue.

Microscopic sections showed that the numerous small, regular nodules which were observed grossly were composed of groups of large, hyperchromatic liver cells derived by regeneration from cells about the portal triads which had escaped destruction during the acute stages of injury. The portal triads, instead of the central veins, now occupied the centers of most of the new pseudo-lobules (Figs. 2, 7, and 12); and the central veins with collapsed stroma and necrotic debris from the central portions of the original lobules, and the newly formed fibrous tissue were now compressed between the peripheral margins of the newly formed nodules of liver cells. This series of events was repeated in whole or in part, although massive necrosis of cells usually occurred only at the beginning of pyridine treatment. Finally it became difficult to relate the architectural pattern to that originally present. In addition to the increase in fibrous tissue, infiltration by lymphocytes and polymorphonuclear leukocytes was observed in some areas.

Some animals on diet 1 containing pyridine developed well advanced cirrhosis in 1 month, and the liver cells of these animals showed extensive fatty changes (Fig. 4). Most of the animals that received diet 3 with pyridine for 2 months or more exhibited unquestionable cirrhotic changes, with disorganization of the normal lobular architecture, not accompanied by much fat (Fig. 7). In most of the latter animals, the fibrosis, while generalized, was not very abundant.

Calcium-Containing Bodies in Necrotic Liver Cells. In the chronically injured livers, collections of hepatic cells containing closely packed granules or globules in the cytoplasm, staining dark brown with alum hematoxylin, were much more striking than in the earlier stages of injury, although they were not observed in every case. These cytoplasmic bodies were not highly refractile and not readily visible in unstained sections. They were smoothly oval, variable in size, and could be distinctly seen individually only with high magnification (Fig. 15). Necrotic liver cells, often still in cords, frequently remained as somewhat hyaline, eosinophilic masses without nuclei or with only nuclear fragments (Figs. 11 and 12), and the cells containing the dark particulate bodies were confined for the most part to the peripheral margins of these old necrotic areas (Figs. 12 to 15). They closely surrounded the nodules of viable hepatic cells, and, when examined under low magnification, appeared to form crescents or rings about the nodules (Figs. 12 and 13). The cells containing the bodies stained very

prominently with silver (Fig. 13) when sections were placed in silver nitrate solution and exposed to ultraviolet light, according to von Kossa's method for staining calcium deposits.* After treatment of the sections with hydrochloric acid, the bodies were no longer demonstrable. The bodies were not stained by nuclear stains of the methylene blue type. Neither the bodies nor other substances in the sections were acid-fast, or stained by fat stains after use of fat solvents, or fluorescent with ultraviolet light. The individual globular bodies often appeared darker at the periphery than at the centers.

Large Regenerative Nodules. Examination of the sections of the tumor-like nodules which developed in the later periods of the study revealed masses of large, compact, deeply staining cells with large, hyperchromatic nuclei (Fig. 16). The nodules were sharply circumscribed and no evidence of invasion or metastasis has been noted in the animals thus far autopsied. The cells composing the nodules, while different in appearance from the remaining liver cells, were evidently derived from parenchymal cells. Slight bile duct hyperplasia was noted occasionally, but nothing resembling a malignant neoplasm of this origin was seen.

Later Renal Lesions. Grossly, the kidneys from animals receiving prolonged treatment exhibited irregular surfaces, with shallow depressions and raised areas. Sections showed that the degenerative lesions were usually less marked than in the early stages, but there were additional changes of a more chronic nature. Destruction of parenchymal cells followed by regeneration occurred, often with some distortion and disruption of normal architecture, and a moderate number of calcified tubules were seen. Frequently the original epithelial lining of the tubules appeared to have sloughed into the lumina and to have been replaced by new epithelium. The tubules showed much more than normal variation in size, and many exhibited closely placed, hyperchromatic nuclei, with considerable variation in size, shape, and relative position in the cells (Figs. 8 and 9). Mitotic figures were noted frequently (Fig. 10).

Along with this destruction and regeneration of tubular cells, there appeared many dilated tubules with flat, atrophic-appearing epithelium (Fig. 8), which in extreme cases had the appearance of endothelium. The dilatation perhaps occurred earliest in the distal segments of the nephrons and in the collecting tubules but also involved the proximal

* It should be recorded that many of the specimens were fixed in formalin which had been neutralized by calcium carbonate. However, livers fixed in redistilled alcohol also showed the calcium bodies.

convoluted tubules and loops of Henle. The relationship of this process to the other changes in the kidney was not entirely clear.

Proliferation of connective tissue in limited areas was sometimes seen, but a generalized increase in fibrous tissue of a significant degree, such as occurred in the livers, was not observed. Occasional areas of granulomatous interstitial inflammation, some containing many eosinophils, were noted. In the regions of some of the angular calcium plaques of the type frequently seen in areas of old renal necrosis, there were necrotic cells containing brown-staining globular bodies similar to those seen in the livers.

Influence of Mode of Administration on the Effects of Pyridine

Because of the observation that some animals survived amounts of pyridine in single injections greater than the total amounts ingested by others which died after several days on the pyridine-containing diets, pyridine was administered by injection to a limited number of animals. The majority of a group of rats survived throughout the experimental period of 3 weeks when given twice daily, in subcutaneous injections, more pyridine than was usually consumed in the same periods by animals receiving pyridine in the diet.

The effects of pyridine given in equal amounts during each 24-hour period, by feeding in the diet, by daily subcutaneous injection, and by daily injection by stomach tube during a fasting period were then compared in three groups of rats using the paired-feeding technic, and the rats receiving the pyridine-containing diets died before the corresponding animals of the other groups in almost every case, and showed more extensive hepatic and renal injury.

DISCUSSION

Damage produced by the diets containing pyridine was apparently limited, for the greater part, to the liver and kidney, with injury of lesser degree to the spleen and perhaps to the lung and certain other organs. Except for narcosis with large doses, the effects upon the nervous system, which have been described for other species, were not observed.

The extent of the acute hepatic necrosis, even in many animals which survived, was remarkable. Whether this necrosis was produced by a direct action of the toxic agent on the cells, or was secondary to interference with the blood supply to large portions of the central parts of the lobules caused perhaps by cellular swelling, such as has been described by others under different circumstances,¹¹ was not determined. The survival of the cells about the portal triads with

regeneration from these areas, together with the observations that in most cases the necrosis was either extensive or practically absent, and that animals which survived extensive necrosis often seemed to be resistant, at least for a time, to the further development of necrosis, suggested the latter possibility.

Rather marked reduction in fatty changes and fibrosis, with no significant reduction in necrosis, caused by increasing the choline and casein content of the diet at the same time that the pyridine level was being increased, suggested, perhaps, that the necrosis and the fatty changes and fibrosis were not results of the same single disturbance. The experiments did not distinguish clearly between the effects of choline and casein, but it seemed likely that the increase in the choline level was principally responsible for the decrease in fat and fibrous tissue, while the increase in the resistance to necrosis probably was due to the change in casein content. It is possible that dietary inadequacies of these substances were contributory causes of the lesions observed with pyridine on the first two diets, although no extensive pathologic changes occurred in the control animals on these diets.

The reversal of the normal relationship between the lobules of liver cells and the blood vessels and bile ducts, with fibrosis across the central portions of the original lobules, which occurred following the acute stages of injury, was striking. It recently has been shown by injections of India ink into the portal and hepatic veins that the fibrous trabeculae in dietary and carbon tetrachloride cirrhosis in the rat are around hepatic veins rather than periportal.¹² The question arises whether the lesions observed in the present study should not be considered examples of post-necrotic scarring rather than diffuse hepatic fibrosis or cirrhosis. The difference in the development and significance of these lesions has recently been discussed by Himsworth and Glynn.¹³ Scarring in old areas of necrosis was quite prominent, particularly in the animals on diet 3, but whether all of the fibrosis occurred as a result of necrosis was not determined. The livers in the late stages of injury presented, in addition to some areas of obvious post-necrotic scarring, finely granular surfaces (Fig. 6) and fairly uniform involvement of all lobules by the fibrotic process. Whatever was the mechanism of the fibrosis, the lesions had all of the features which are usually considered characteristic of cirrhosis. Observations on the pyridine-treated animals will be continued to determine to what extent the lesions are repaired, and to see if the large hepatic nodules progress to malignant tumors.

The sequence of pathologic events observed in the kidneys of the

animals receiving pyridine was similar in many respects to that in the livers, in so far as the epithelial cells were concerned. The responses of the mesenchymal elements differed, however, in that there was no significant generalized increase in fibrous tissue.

The causes of the dilated tubules in the chronically injured kidneys were not wholly apparent. Some tubules probably were obstructed by fibrosis or calcification resulting from injury, and it is possible that others were obstructed by albuminous material and cellular debris. Localized and generalized cellular swelling, resulting in encroachment on the lumina of blood vessels and tubules, might conceivably have contributed to the production of both necrosis and obstruction.

The renal lesions were somewhat similar to, but much greater in degree, than those observed by Baxter and Ashworth¹⁴ in human cirrhosis. It was not determined with certainty whether the renal injury was produced independently or occurred as a result of the hepatic injury. As in the case of the human lesions referred to above, it is possible that shock and vasoconstrictive renal ischemia played a part in the production of renal damage.

Apparently there have been no descriptions of calcification of the type observed in the present investigation, in reports of somewhat similar hepatic injury produced by other means. Deposition of calcium in the liver, except in certain cysts, abscesses and tumors, is rare. Even in generalized calcinosis, the liver is infrequently involved. Whether there was something specific about the pyridine injury that was responsible for the calcium deposition is not known. Since the animals also had extensive renal injury with nitrogen retention, calcification may have been favored by high levels of blood phosphate. Generous amounts of vitamin D were used in the diet, but not enough to produce calcification in normal animals. Determinations of blood calcium and phosphorus, and of the phosphatase content of the injured cells might have shed light on the mechanisms of the calcification. The calcium deposition differed from that usually seen in necrotic tissue in that it was confined to individual cells, and the calcium was deposited in the form of globules, or, perhaps, about the surfaces of preformed cytoplasmic bodies. The bodies resembled in size and shape those described by Opie¹⁵ in livers injured by butter yellow, formed by the deposition of ribonucleic acid about the mitochondria.

The principle involved in the greater toxicity of pyridine when continuously present in the diet than when given in infrequent injections may be of wider and possible practical significance in the causation of hepatic injury other than that produced by pyridine. It seems pos-

sible that the difference in effects observed with the different methods of administration was due to the more continuous presence of pyridine in the tissues, when ingested at frequent intervals in the food, or, less likely, to the formation by intestinal bacteria of a substance more toxic than pyridine itself. Other substances of low toxicity, which do not produce hepatic injury by the usual methods of administration, might conceivably behave in a manner similar to pyridine if taken in the diet or if continuously formed in the body or in the intestinal tract, and result in hepatic or renal damage even when present in low concentrations.

SUMMARY

Pyridine or pyridine citrate, when incorporated in diets which did not themselves produce pathologic changes during the experimental period, caused acute hepatic and renal injury, followed by regenerative changes, cirrhosis, and chronic renal injury.

Increasing the choline (and casein) content of the diet at the same time that the pyridine level was also being increased, caused a marked reduction in fatty changes and fibrosis without any significant reduction in the severity and extent of the acute necrosis.

Large tumor-like nodules were observed in some of the livers, but no evidence of invasion or metastases was seen.

In parenchymal cells at the edges of old necrotic areas in the livers, there were accumulations of oval cytoplasmic bodies which stained dark brown with hematoxylin and gave the histochemical reactions of calcium.

Amounts of pyridine which produced death due to hepatic and renal injury when fed in the diet apparently were much less toxic when administered by infrequent subcutaneous injection or by stomach-tube.

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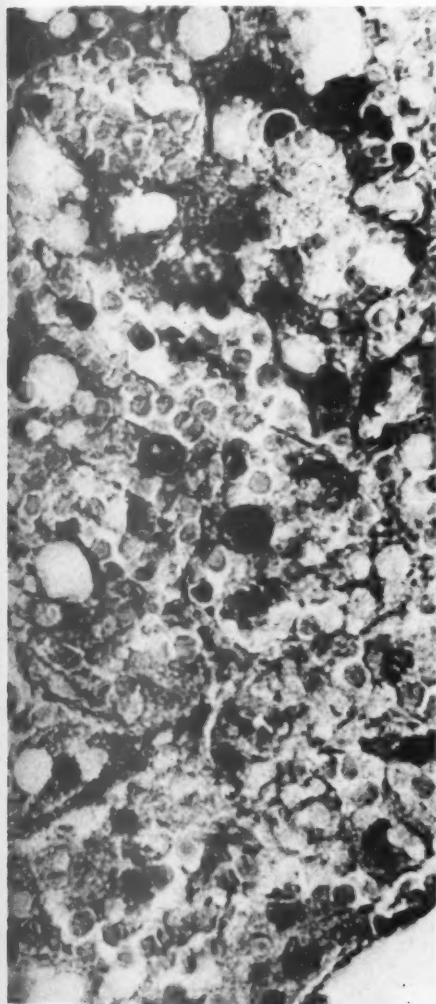
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DESCRIPTION OF PLATES

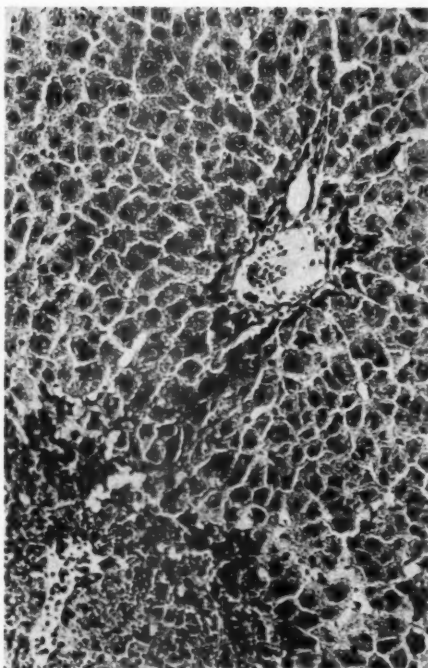
PLATE 90

- FIG. 1. Necrosis of cells about the central vein in the liver from a rat which died after 16 days on diet 1 plus 0.2 per cent pyridine. Dissolution of cells with nuclear fragmentation may be noted. Many necrotic cells remain as shells, some of which are full of red blood cells. Surviving cells in the peripheral portions of the lobule contained fat globules. Hematoxylin and eosin stain. $\times 580$.
- FIG. 2. Regeneration of a liver nodule from the cells about a portal triad, in an animal sacrificed after 35 days on diet 2 plus 0.2 per cent pyridine. The portal triad is at the center of the new pseudo-lobule, with the necrotic area at the periphery. Dark-staining cells are seen at the edge of the necrotic zone. This animal almost died in the early stages of the experiment, but after the initial illness was able to continue on the same diet without serious results. Hematoxylin and eosin stain. $\times 110$.
- FIG. 3. Degenerative changes in the epithelium and tubular casts in the kidney from a rat dying after 3 days on diet 1 plus 0.1 per cent pyridine. Hematoxylin and eosin stain. $\times 110$.
- FIG. 4. Cirrhosis of the liver with extensive fatty infiltration. The rat was sacrificed after 35 days on diet 1 with 0.1 per cent pyridine. For comparison with Figure 7. A photograph of the liver from which this section was taken is shown in Figure 5. Hematoxylin and eosin stain. $\times 110$.

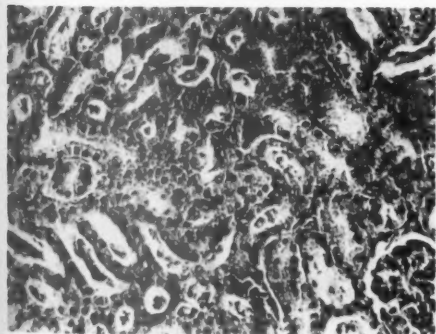




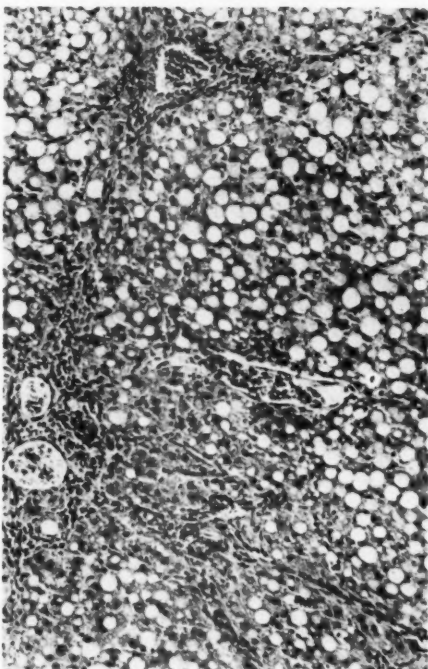
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Hepatic and Renal Lesions Following Pyridine

PLATE 91

FIG. 5. The organs on the left side of the photograph are from the same rat as the section shown in Figure 4. The kidneys are comparatively large. This animal, like most of those on diet 1 plus pyridine, grew poorly, and the small size of the testes and heart was probably not a specific effect of the pyridine. On the right are shown the organs of a litter mate which received the same diet, plus 0.5 per cent of added methionine, for the same length of time. $\times 1$.

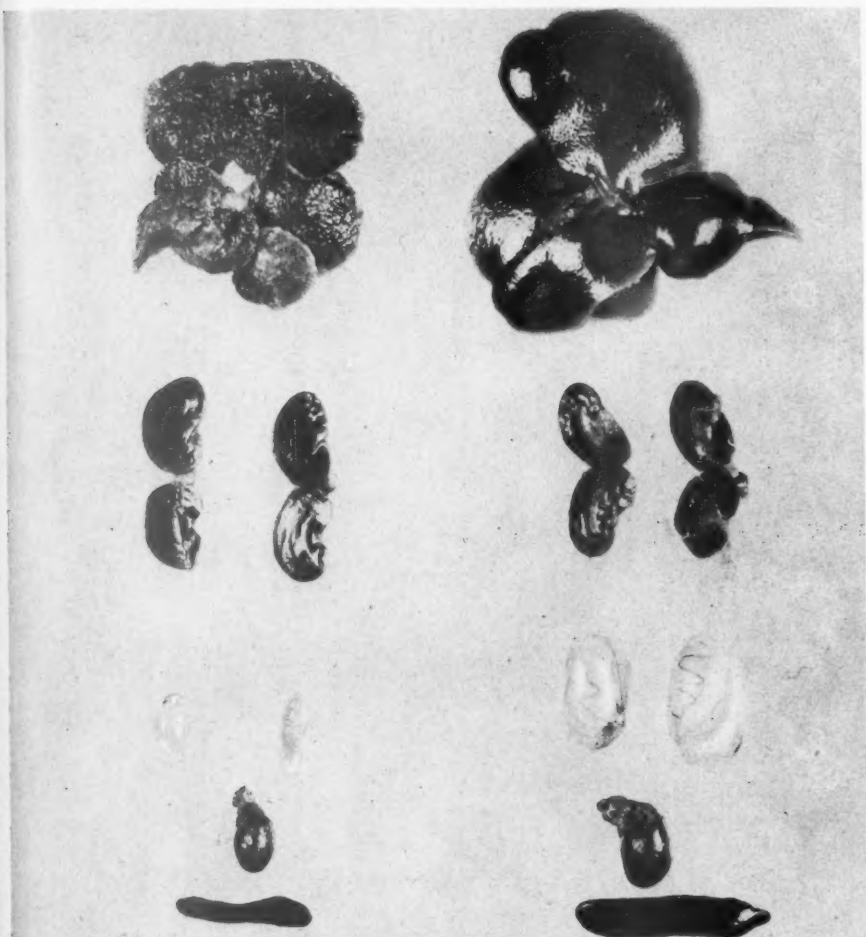
FIG. 6. Photograph of liver, kidneys, and spleen of a rat which died after 100 days on diet 3 plus 0.7 per cent pyridine citrate. The surfaces of the liver are finely granular. A section of this liver is shown in Figure 7. $\times 7/10$.

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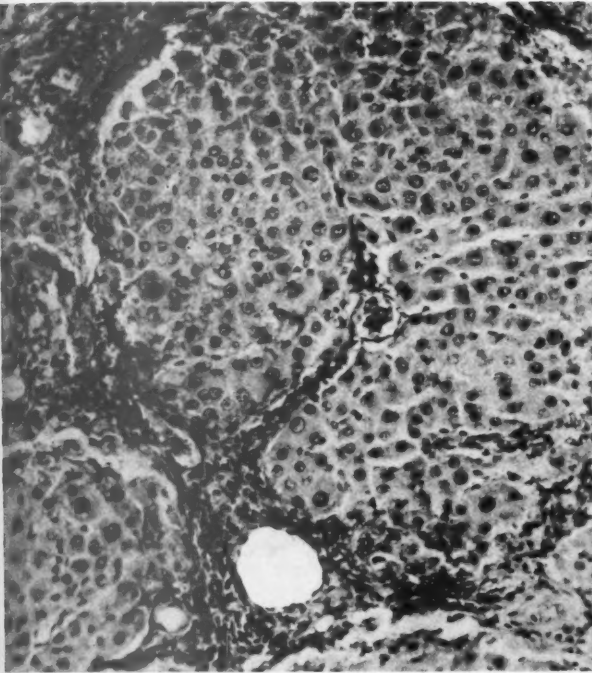
Hepatic and Renal Lesions Following Pyridine

PLATE 92

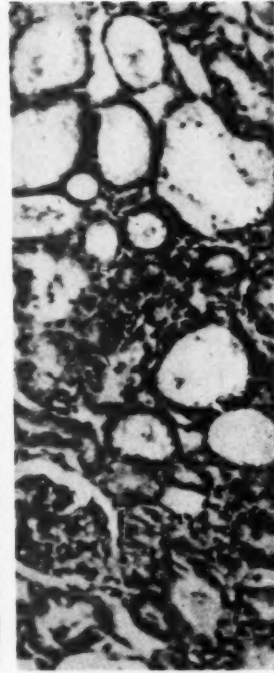
- FIG. 7. Section from the liver seen in Figure 6, showing cirrhosis with little fat. Extensive necrosis and regeneration have occurred, and again the characteristic structures of the portal triad were seen at the centers of many of the pseudo-lobules. Although the fibrous tissue was not abundant in most areas, it involved all of the lobules fairly uniformly. Hematoxylin and eosin stain. $\times 150$.
- FIG. 8. Kidney from the same animal as Figure 7, showing chronic changes characterized by regeneration of epithelium and tubular dilatation. Hematoxylin and eosin stain. $\times 150$.
- FIG. 9. Another area of the kidney shown in Figure 8. The original epithelial lining of the tubule apparently has been sloughed into the lumen, and the tubule is now lined by newly regenerated cells. The nuclei are closely placed, and vary in size, shape, and relative position in the cells. Hematoxylin and eosin stain. $\times 300$.
- FIG. 10. One of the fairly numerous mitotic figures noted in the kidney from a rat on diet 3, with 0.34 per cent pyridine citrate for 2 months and 0.7 per cent pyridine citrate for an additional 2 months. Animal was found in shock and was autopsied. Hematoxylin and eosin stain. $\times 1000$.
- FIG. 11. Nodule of liver cells from a rat which died after receiving diet 3 with 0.7 per cent pyridine citrate and choline *ad libitum* for 60 days. Most of the cells in this nodule were necrotic, without stainable nucleus or with only nuclear fragments. Various stages of calcification of the necrotic cells are evident. Hematoxylin and eosin stain. $\times 300$.



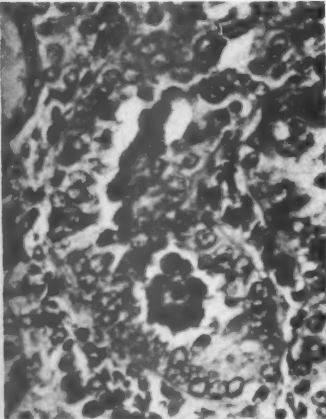
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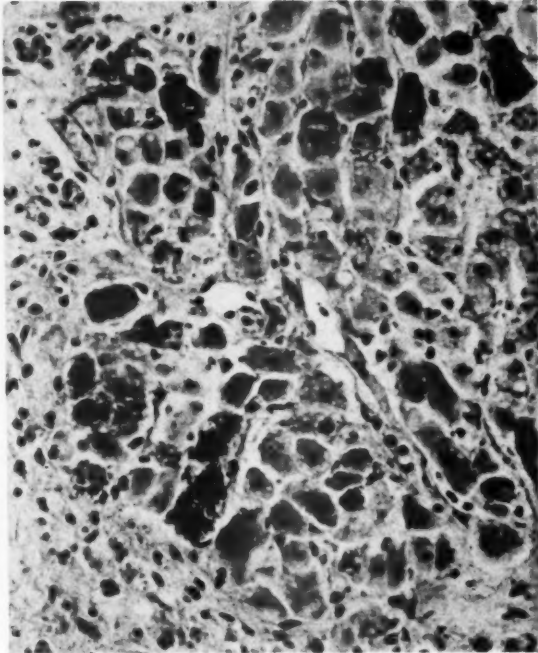
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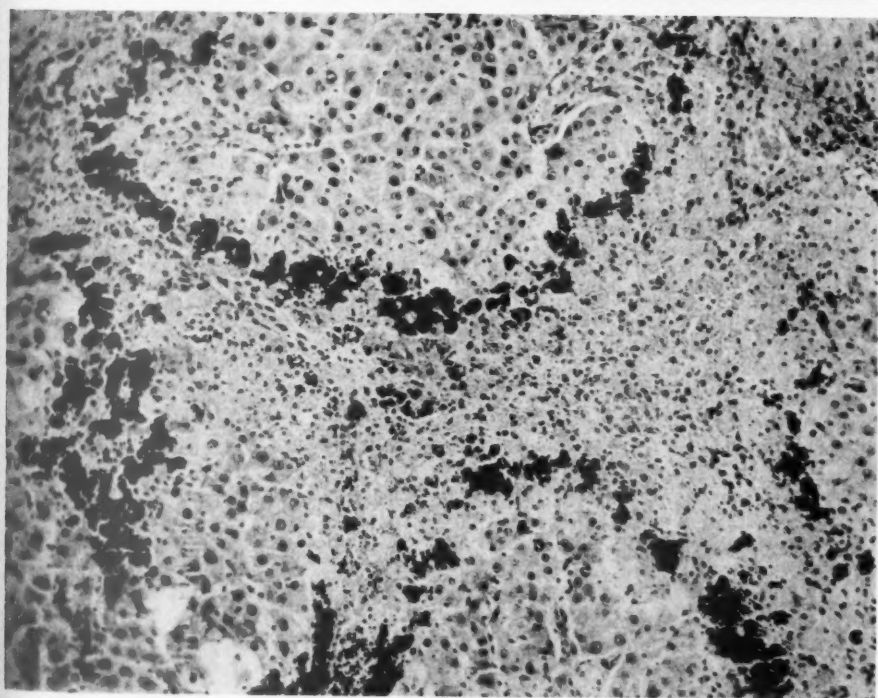
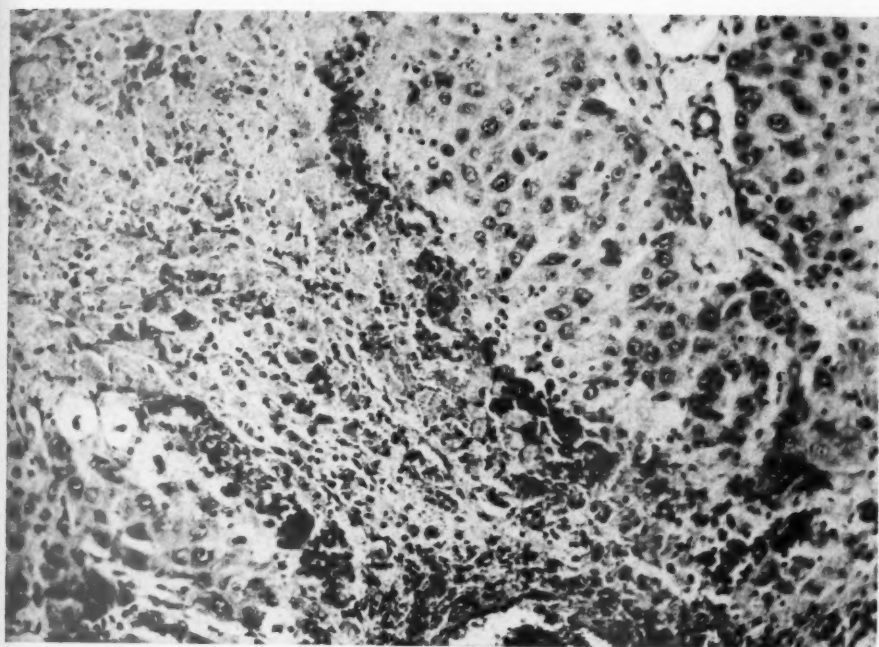
Hepatic and Renal Lesions Following Pyridine

PLATE 93

FIG. 12. Liver from the same animal as Figure 10, showing the characteristic location and distribution of the calcified cells. They occurred chiefly at the edges of the old necrotic areas and closely surrounded the nodules of viable cells. Here again a bile duct and other structures of a portal triad are seen at the center of a nodule of liver cells. Hematoxylin and eosin stain. $\times 150$.

FIG. 13. A section of the same liver as in Figure 12, showing the calcium deposits stained black with silver. The preparation was made by placing the section in a solution of silver nitrate and exposing it to ultraviolet light, according to the method of von Kossa. $\times 150$.





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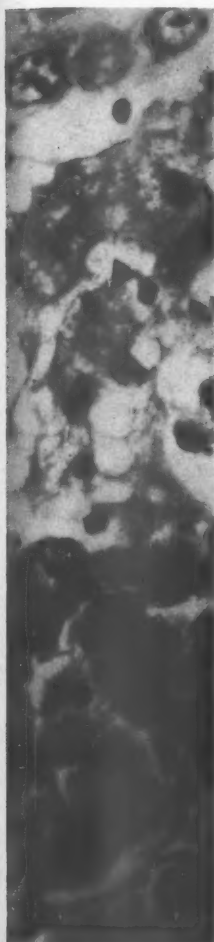
Hepatic and Renal Lesions Following Pyridine

PLATE 94

- FIG. 14. A section of the same liver as used for Figures 12 and 13, showing the transition, at the edge of an old area of necrosis, from the normal liver cells at the top of the section to the eosinophilic remains of cells at the bottom. The cells in the intermediate zone are calcified. Hematoxylin and eosin stain. $\times 700$.
- FIG. 15. Another area of the same section as for Figure 14, showing the various forms of cellular degeneration observed in passing from the fairly normal cells of a hepatic lobule at the upper left to the hyalinized remains at the lower right. The early stages of calcium deposition are well shown. Some of the calcium bodies were in focus and are seen as smoothly oval globules of varying sizes. The bodies seemed to be darker at the periphery than at the centers in many cases. Hematoxylin and eosin stain. $\times 1000$.
- FIG. 16. Section showing the edge of a large tumor-like nodule of regenerative hepatic cells from the same liver as Figure 11. The cells are of large size. The dark-staining liver cells embedded in the fibrous tissue are calcified. Hematoxylin and eosin stain. $\times 100$.



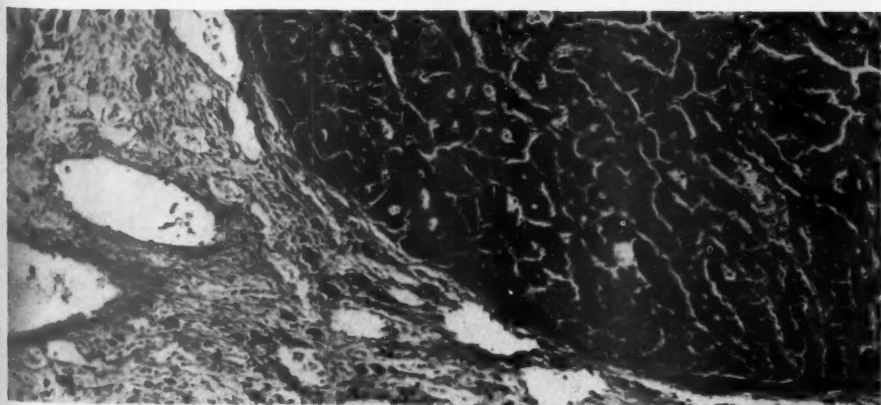
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Hepatic and Renal Lesions Following Pyridine

BILIARY XANTHOMATOSIS (XANTHOMATOUS BILIARY CIRRHOSIS)*

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Boston 15, Mass.)

Ten years ago Thannhauser and Magendantz¹ referred to a peculiar histologic change in the liver which they called "xanthomatous biliary cirrhosis." They distinguished this entity clinically and anatomically from other types of biliary cirrhosis associated with simple obstruction of the common bile duct. The specific histologic lesion, and the one on which the term was based, consisted of xanthoma cells and increased fibrous tissue in the walls of the intrahepatic system of bile ducts. This xanthomatous deposition in the liver, like xanthomata in other parts of the body, was considered to be a local manifestation of a hereditary and systemic disorder which they called essential hypercholesteremic xanthomatosis. The term "xanthomatous biliary cirrhosis" was used at the same time to designate a clinical syndrome in which xanthomatous change in the liver was but one of its most conspicuous features. This syndrome was characterized by chronic jaundice, an enlarged liver, hypercholesteremia, and, above all, xanthomatosis. It is important at this point to emphasize the double meaning that was assigned to "xanthomatous biliary cirrhosis," and it must be clearly understood that the changes throughout the liver were considered to be but one component of a systemic disease and not the cause of the syndrome. This has been confusing for it is always ambiguous to use the same term to denote a specific anatomic lesion, and to designate a clinical syndrome when that particular anatomic lesion is not regarded as the cause, but merely an integral part of the syndrome.

MATERIAL FOR STUDY

Through the interest and cooperation of Dr. Thannhauser, it has been my privilege to study adequate biopsy sections from the livers of 4 patients whom he selected as showing the signs and symptoms of the syndrome "xanthomatous biliary cirrhosis." Each of the 4 patients had been jaundiced for months and each had an enlarged and palpable liver. The cholesterol levels of the blood were above normal and each

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patient manifested the peculiar xanthomatous changes on the hands and body which are so characteristic of this disorder. At the time of biopsy, the extrahepatic biliary tract was normal. Two of these patients subsequently died at intervals of about 1 and 2 years after the original specimens were obtained. This enabled a follow-up study to be made and afforded the opportunity to compare early and late lesions.

BIOPSY FINDINGS

It was naturally anticipated that collections of xanthoma cells would be found in the walls of the bile ducts since this had been described as the specific histologic lesion peculiar to this clinical disorder. The findings in all four specimens were similar, but they were very disappointing, for in not one of the sections were xanthoma cells found in the walls of the bile ducts. In other words, there was no evidence in any of the biopsies of a lesion that one could call "xanthomatous biliary cirrhosis." Instead, there was a chronic proliferative and exudative inflammatory reaction in the portal areas which was most concentrated about the junctional ducts (canals of Hering) and terminal bile ducts (interlobular bile ducts or cholangioles) at the periphery of the lobules (Fig. 1). The portal areas were larger, broader, and longer than usual and often fused with one another to form rings of perilobular fibrosis (Fig. 2). Inflammatory granulation tissue extended into the periphery of the lobules. It blocked canaliculi, destroyed liver cells, and collapsed many sinuses (Figs. 3 and 4).

The larger bile ducts were patent and empty. The small interlobular bile ducts were very difficult to find and in many of the portal areas there were none. The junctional ducts, which in a normal liver are inconspicuous, were numerous, elongated, branching, and tortuous. Some were dilated and filled with bile, others were collapsed and empty. None of the ducts contained leukocytes, although several types of inflammatory cells, including lymphocytes, plasma cells, histiocytes, neutrophils, and occasionally eosinophils, richly infiltrated the surrounding granulation tissue.

The lobular pattern of the liver was well preserved and most central veins bore a normal relationship to the surrounding liver parenchyma. Large and sometimes lamellated bile casts were fairly numerous within the lobules (Fig. 5). These distended the canaliculi and often damaged the bordering liver cells. For the most part the liver cells were healthy; a few contained fat droplets; and some lying in the field of inflammatory reaction about the portal areas showed degeneration and necrosis. There were few mitotic figures and the presence of closely

packed clusters of small cells near the periphery of the lobules suggested a moderate degree of liver cell regeneration (Fig. 6).

In one of the four specimens the damage to the parenchyma was greater than in the others. In this there were fields in which the inflammatory reaction had cut deeply into the centers of the lobules (Fig. 7). In this way central veins became linked with portal areas and large and small islands of liver cells became isolated from the body of the parent lobules. These islands, in turn, seemed to melt away to become replaced by inflammatory granulation tissue (Fig. 8). In one area, an entire lobule had been destroyed and was now substituted by fibrous tissue.

The one fundamental histologic finding common to all four specimens was a chronic inflammatory reaction in the interstitial portal areas. It began as a chronic pericholangiolitis and spread into the peripheral zones of the adjacent lobules.

AUTOPSY FINDINGS

To understand cirrhosis it is necessary to know it in all its stages, and the opportunity to compare sections from the livers of patients during life with sections obtained months and years later in the course of autopsies carries with it a definite responsibility, particularly when the liver is the seat of a little understood and very debatable disease process.

Grossly, the livers of the 2 patients who were autopsied were large, firm, and cobbled. They weighed 2600 and 2800 gm., respectively. On section the texture was coarse and the parenchyma bile-stained. There were no depositions of cholesterol in the mucosa of the gallbladder or in any of the bile ducts. There were no concretions and, finally, there was no suggestion of any type of extrahepatic biliary obstruction.

The histologic features were much more complex and confusing than in the earlier material. In a few areas the central veins, central zones, and mid-zones were still recognizable, but for the most part the normal lobular pattern was now severely distorted. Bands of fibrous tissue divided the parenchyma into irregular and uneven nodules. The portal areas were composed of wide and communicating bands of connective tissue that extended deeply into the parenchyma and occasionally replaced whole lobules (Fig. 9). The parenchyma was composed of irregular cords of liver cells separated by edema fluid, dilated sinuses, and fibrous tissue. Here and there, the Kupffer cells were large, swollen, and filled with lipids, and nests of these cells occasionally distended the sinuses and compressed the adjacent trabeculae (Fig. 10). There was

much bile stasis, with bile in the canaliculi, liver cells, and sinuses. The larger bile ducts were collapsed and empty. The terminal ducts were inconspicuous and embedded in fibrous tissue. In none of the ducts was there either an inflammatory exudate or any evidence of xanthomatosis.

The large size of the liver, the extensive fibrosis, the fragmentation of some lobules and the total loss of others, the nodules of regenerated liver tissue, the compression and interruption of liver cords by fibrous tissue, the bile stasis, the intralobular lipid deposition, and, finally, the presence of a still active chronic inflammatory reaction in portions of the interstitial tissue all combined at this late stage to form a very confusing histologic picture. If such a damaged liver were to be seen for the first time, without a previous biopsy, its pathogenesis would be difficult to unravel and its classification would be uncertain.

COMMENT

Because the etiology of this disease of the liver is still unsettled, the descriptive term "pericholangiolitic biliary cirrhosis" is suggested. This emphasizes the inflammatory nature of the process and at the same time clearly denotes the exact site of the earliest lesion. It resembles other types of biliary cirrhosis such as the obstructive and the cholangiolitic, but from each of these it may, particularly in the early stages, be readily distinguished. There now seems to be little justification for referring to this change in the liver as xanthomatous biliary cirrhosis because this type of cirrhosis may exist in the absence of xanthomatosis.

"Pericholangiolitic biliary cirrhosis" with its destruction of terminal bile ducts and liver cells, and its proliferation of granulation tissue and subsequent fibrosis explains the retention and regurgitation of bile and the appearance clinically of jaundice. It will also account for the enlargement of the liver. Could it alone be responsible for the great increase of cholesterol and for the appearance of xanthomatosis? This is a debatable question. It must be admitted that bile stasis alone is rarely the cause of xanthomatosis. Yet, in this small collection of 4 patients and in all other cases that have been reported (Table I), long-standing jaundice consistently has been the earliest symptom. This implies that an interference in the normal secretion and elimination of bile, although perhaps not the only factor, is at least essential. In this respect, it is of interest to note that the syndrome is found almost exclusively in females of about 40 years of age. This suggests that constitutional factors, particularly those associated with age and sex,

TABLE I
Cases That Have Shown the Clinical Syndrome of "Xanthomatous Biliary Cirrhosis" in which the Liver Was Examined

| | Date | Author | Sex | Age | Examination | Diagnosis |
|----|------|--|-----|-------|-------------|--------------------------------------|
| 1 | 1869 | Murchison ³ | M | 41 | Autopsy | Cirrhosis |
| 2 | 1873 | Fagge ³ | F | Adult | Autopsy | Cirrhosis |
| 3 | 1873 | Moxon ⁴ | M | 32 | Autopsy | Stricture of bile duct* |
| 4 | 1873 | Pye Smith ⁵ | F | 49 | Autopsy | Gallstone obstruction* |
| 5 | 1905 | Futcher ⁶ | F | 39 | Laparotomy | Gallstone obstruction* |
| 6 | 1905 | Futcher ⁶ | F | 39 | Autopsy | Gallstone obstruction* |
| 7 | 1905 | Futcher ⁶ | F | 42 | Laparotomy | Hypertrophic cirrhosis |
| 8 | 1908 | Pinkus and Pick ⁷ | F | Adult | Autopsy | Hypertrophic biliary cirrhosis |
| 9 | 1909 | Posner ⁸ | F | 37 | Laparotomy | Cirrhosis |
| 10 | 1911 | Chvostek ⁹ | F | 47 | Autopsy | Hypertrophic biliary cirrhosis |
| 11 | 1924 | Weidman and Freeman ¹⁰ | M | 6 | Autopsy | Stricture of common duct* |
| 12 | 1928 | Dyke ¹¹ | F | 44 | Autopsy | Hypertrophic biliary cirrhosis |
| 13 | 1938 | Thannhauser ¹ | F | 35 | Autopsy | Xanthomatous biliary cirrhosis |
| 14 | 1938 | Thannhauser ¹ | F | 32 | Laparotomy | Xanthomatous biliary cirrhosis |
| 15 | 1938 | Montgomery ¹² | F | 29 | Laparotomy | Postoperative stricture* |
| 16 | 1938 | Montgomery ¹² | F | 43 | Laparotomy | Postoperative stricture* |
| 17 | 1938 | Montgomery ¹² | F | 48 | Laparotomy | Postoperative stricture* |
| 18 | 1938 | Montgomery ¹² | F | 37 | Laparotomy | Postoperative stricture* |
| 19 | 1944 | Eusterman and Montgomery ¹³ | F | 48 | Autopsy | Cirrhosis |
| 20 | 1945 | Hoffbauer, Evans, and Watson ¹⁴ | F | 62 | Autopsy | Gallstone obstruction* |
| 21 | 1947 | Thannhauser, MacMahon | F | 44 | Biopsy | Pericholangiolitic biliary cirrhosis |
| 22 | 1947 | Thannhauser, MacMahon | F | 38 | Biopsy | Pericholangiolitic biliary cirrhosis |
| 23 | 1947 | Thannhauser, MacMahon | F | 46 | Biopsy | Pericholangiolitic biliary cirrhosis |
| 24 | 1947 | Thannhauser, MacMahon | F | 43 | Biopsy | Pericholangiolitic biliary cirrhosis |

* Cases showing extrahepatic biliary obstruction (10 of 24).

may be important. It would appear that once this type of biliary cirrhosis has become established, it could lead, under certain conditions, to the complete clinical syndrome.

Another question that may be asked is this: Is "pericholangiolitic biliary cirrhosis" the only type of liver disease that may lead to, or be associated with, this clinical syndrome? If one includes all degrees of this clinical disorder the answer is no, since almost half of the cases reported in the literature have been secondary to some form of extrahepatic biliary obstruction, and at least two types of biliary cirrhosis, namely, the obstructive and the cholangiolitic, may be associated with obstruction. It is obvious, then, that there is no basis for considering this syndrome as the manifestation of a single and specific disease of the liver, and, as a corollary, it becomes clear that there is no longer any justification for including this syndrome in the family of "hereditary essential hypercholesteremic xanthomatoses."

It will be misleading to continue to refer to the changes in the liver in this syndrome as xanthomatous biliary cirrhosis, and it may be equally confusing to retain this same name in referring to this peculiar clinical disorder. Therefore, it is suggested that this term be discontinued and that a new term for the syndrome should be selected. Because jaundice has been the earliest clinical symptom, and because xanthomatosis is the most striking feature of the syndrome, the name "biliary xanthomatosis" is suggested. It has been pointed out that the findings in the liver in this disorder may vary. If one could be certain in a particular case that the underlying lesion in the liver was "pericholangiolitic biliary cirrhosis," then the more specific name for the clinical picture could be "pericholangiolitic xanthomatosis." If, on the other hand, some form of extrahepatic biliary obstruction was found to be the primary lesion, the term "obstructive xanthomatosis" would be in order.

I wish to express my sincere appreciation to Dr. S. J. Thannhauser for his encouragement and patience in the preparation of this paper and for the use of this clinical material for publication.

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[Illustrations follow]

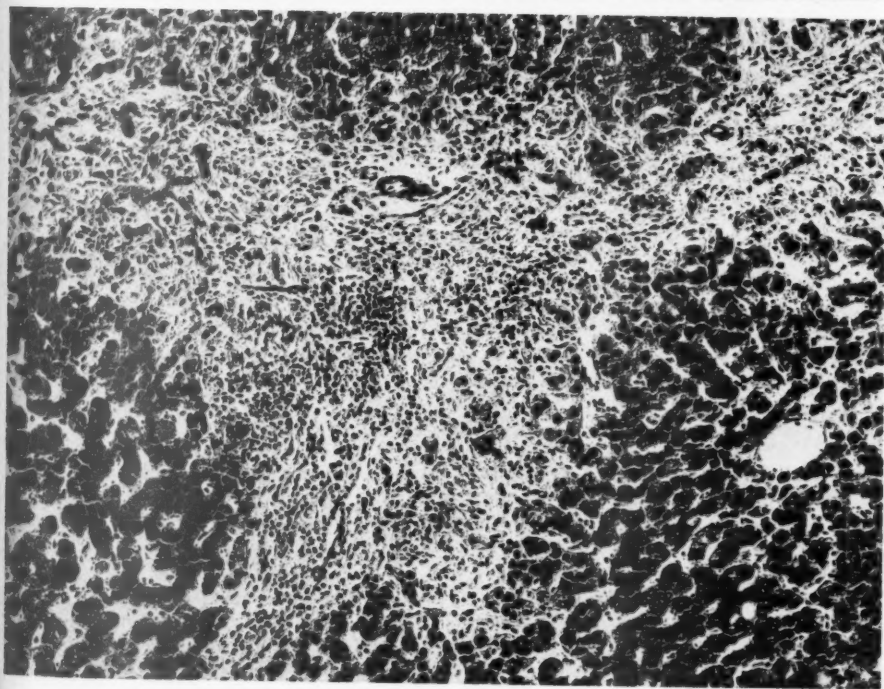
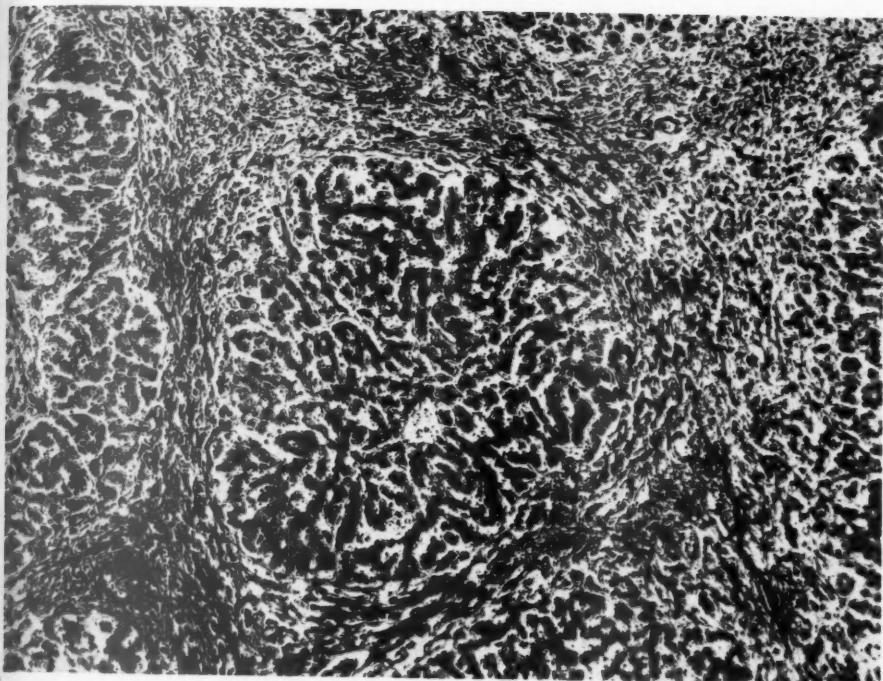
DESCRIPTION OF PLATES

PLATE 95

FIG. 1. Liver. The center of the field is occupied by a single lobule of liver tissue that is sharply demarcated by a band of inflammatory granulation tissue. The central vein lies at about the center of the lobule. The sinuses, moderately dilated, bear a normal relationship to the cords of liver cells. The portal connective tissue contains small arteries and collapsed bile ducts, but neither veins nor lymphatics are recognizable. There is no increase of reticulum within the substance of the lobule. $\times 65$.

FIG. 2. Liver. A wide and roughly T-shaped portal area bordered by the peripheral zones of three adjacent lobules occupies most of the field. The portal vein is collapsed, obliterated, and overgrown by inflammatory granulation tissue. There is a moderately rich infiltration with lymphocytes, histiocytes, a few plasma cells, eosinophils, and polymorphonuclear leukocytes. At several points the inflammatory granulation tissue extends superficially into the adjoining lobules. There are no recognizable interlobular bile ducts in the whole area. The dilated, but intact, central vein in one of the three lobules stands out in striking contrast to the changes in the portal areas and peripheral zones of the lobules. $\times 85$.





MacMahon

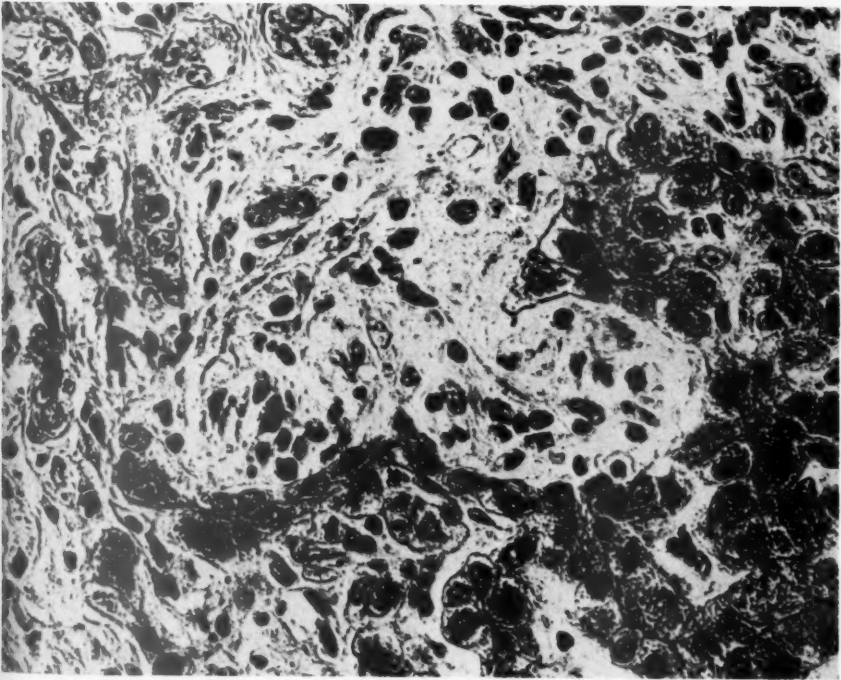
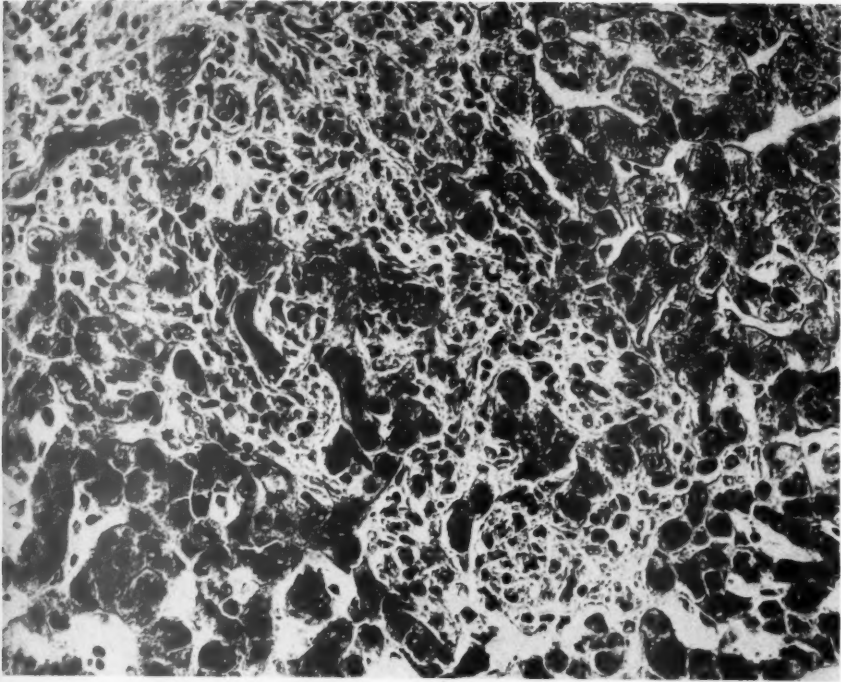
Biliary Xanthomatosis

PLATE 96

FIG. 3. Liver. This field was selected to show a broad, tongue-like expansion of inflammatory granulation tissue cutting into the outer portion of a lobule. Cords and nests of liver cells are completely surrounded by this edematous cellular inflammatory tissue. Sinuses are collapsed or overgrown and in this area are no longer visible. $\times 170$.

FIG. 4. Liver, showing a small field just at the junction of portal connective tissue and liver lobule. This area was selected to show the extension of inflammatory granulation tissue along a sinus into the periphery of a lobule. Parallel cords of liver cells are separated and compressed. The sinuses are obliterated in some areas and compressed in others. There are no interlobular bile ducts in this area. $\times 260$.





MacMahon

Biliary Xanthomatosis

PLATE 97

FIG. 5. Liver. This field was selected to include the outer margin of the peripheral zone of a lobule and the adjoining and very much thickened portal connective tissue. A sharp line separates the two. The most significant finding is the bile stasis with large casts of inspissated bile distending bile canaliculi. The portal area is edematous and is composed of fibrous tissue containing few cords of liver cells. In this field there are no interlobular bile ducts. $\times 265$.

FIG. 6. Liver. This field was selected to show a liver cell in mitosis. The dividing nucleus lies in a compact nest of liver cells in the peripheral zone of the lobule. The involved cell is somewhat larger than the adjoining cells touching it and the chromatin of the dividing nucleus is about equally divided. One-half of the chromatin is suspended in a spindle which is clearly visible toward one end of the cell. $\times 350$.



5



6



MacMahon

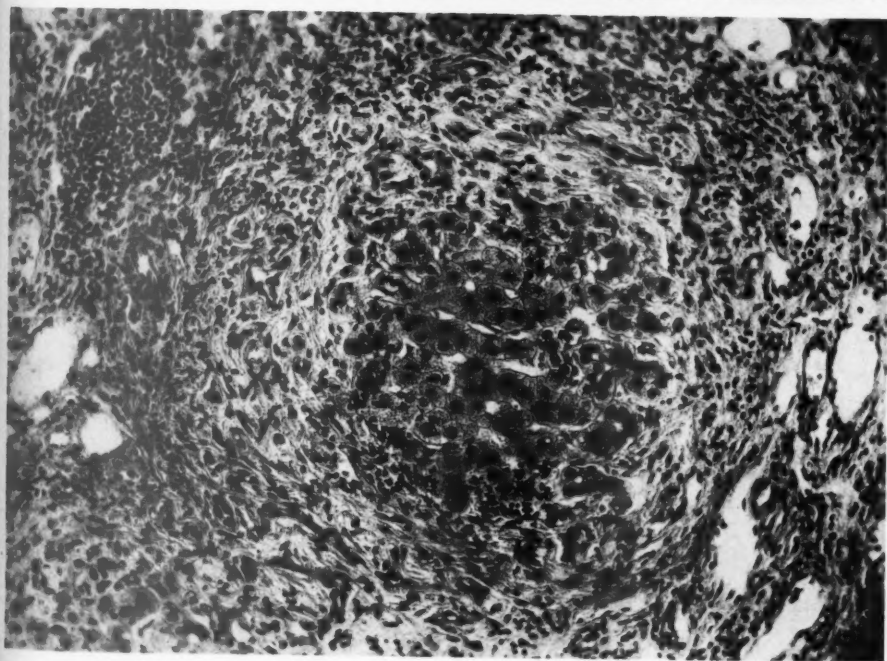
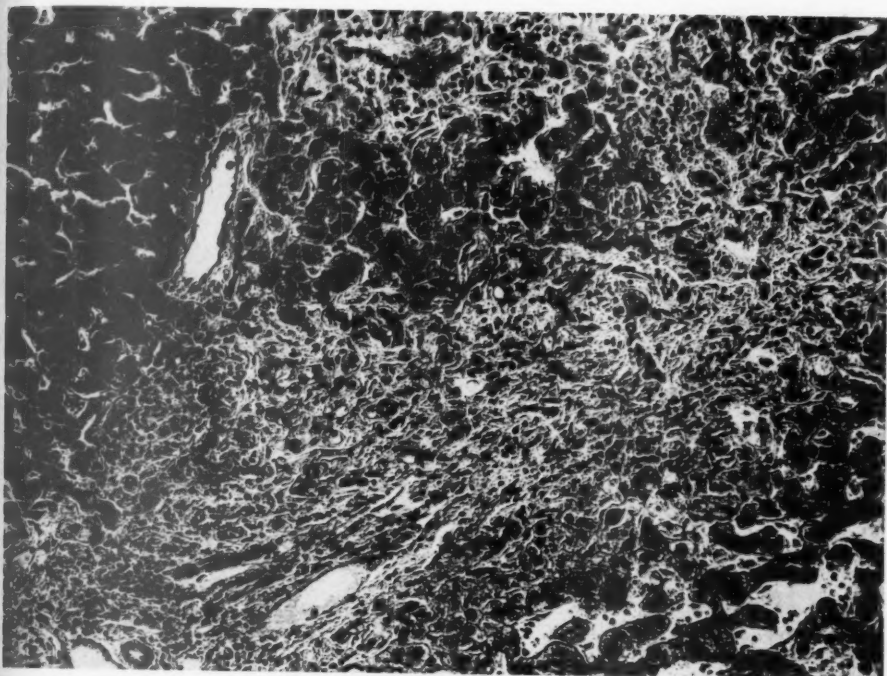
Biliary Xanthomatosis

PLATE 98

FIG. 7. Liver. This field was selected to show encroachment on the lobule by inflammatory granulation tissue. The center of the field is occupied by a nest of compressed liver cells which is bordered on all sides except one by granulation tissue. One surface is bordered by a dilated central vein. Beginning in the portal area, the granulation tissue extends outward along the perisinusoidal spaces as far as the central vein. Many liver cells of the peripheral zone have disappeared. $\times 85$.

FIG. 8. Liver, showing a small compact nest of liver cells—all that remains of a lobule. It is completely surrounded by granulation tissue growing in from the surrounding portal areas. In the area of inflammation, all liver cells have disappeared. $\times 130$.





MacMahon

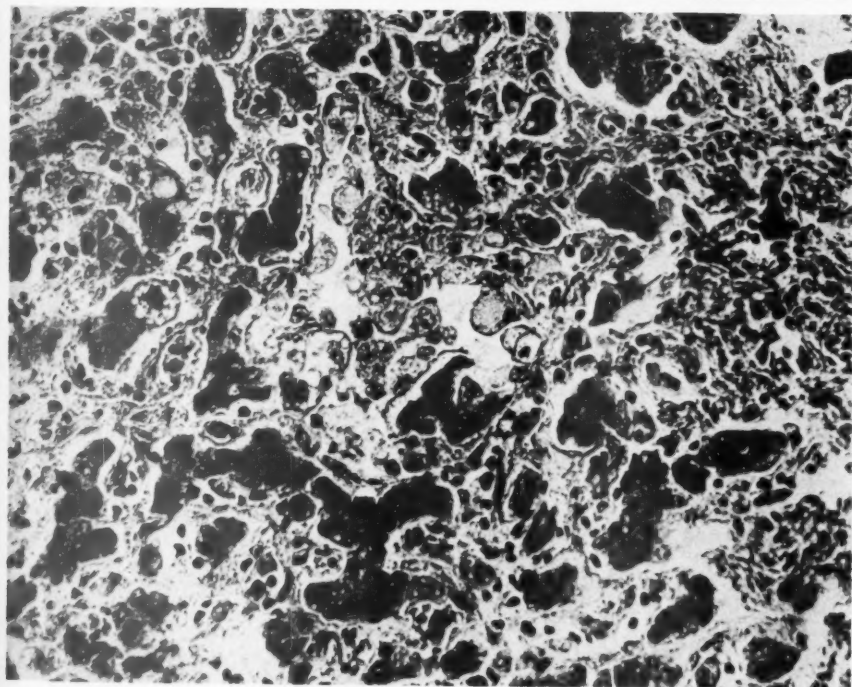
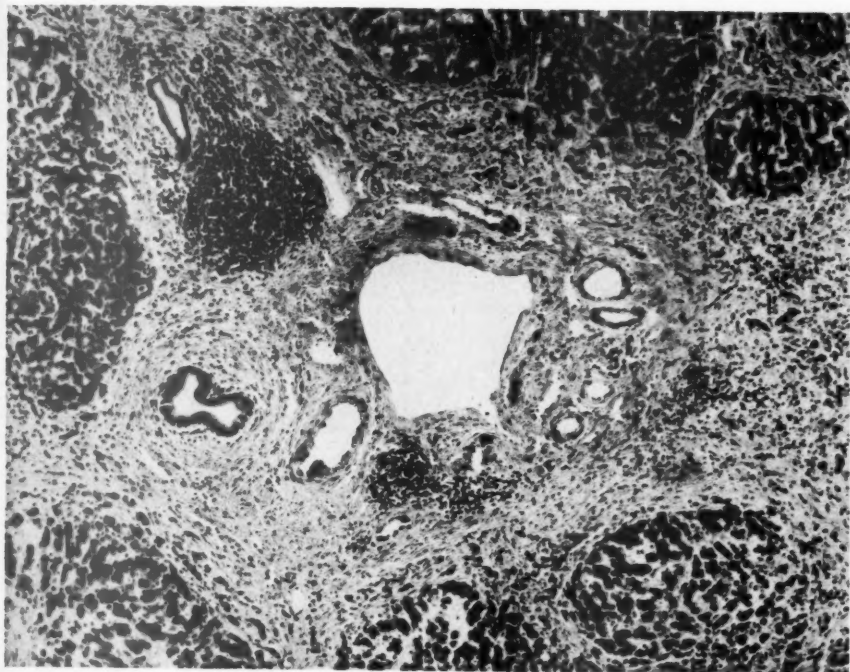
Biliary Xanthomatosis

PLATE 99

FIG. 9. Liver. The center of the field is occupied by a medium-sized portal area. The periphery is represented by portions of the adjacent lobules. The portal vein and its branches are dilated, as are the arteries. The medium-sized bile ducts are empty. There is a great increase in fibrous tissue expanding the entire portal area. There are nests of lymphocytes and narrow and compressed cords of liver cells. A fairly sharp line of demarcation separates fibrous tissue from the parenchyma of the adjoining lobules. $\times 45$.

FIG. 10. Liver. This field was selected from the peripheral zone of a lobule to show collections of lipid-laden endothelial cells within the confines of the lobule. These cells lie in distended sinuses. The cords of liver cells are interrupted, compressed, and distorted. No bile ducts are visible in this area and no xanthoma cells are visible in the adjacent portal connective tissue. $\times 260$.





MacMahon

Biliary Xanthomatosis

CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE
I. THE FORMATION OF "BLISTERS" ON CELLS IN SUSPENSION (POTOCYTOSIS), WITH OBSERVATIONS ON THE NATURE OF THE CELLULAR
MEMBRANE *

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In a study of various tissue cells by means of the phase microscope (PM), a curious formation of "blisters" has been observed on several types of cells in suspension. The circumstances under which this phenomenon has been observed will be given in the present paper, along with certain implications which the findings may have in relation to the nature of the membranes surrounding cells.

THE PHASE MICROSCOPE

The PM equipment, first described by Zernike,¹ makes use of the following principle: If two lightbeams A and B (Text-Fig. 1) of the same wave length (λ) pass through two very thin glass plates, Ga and Gb, of the same thickness and refractive index, their original wave length becomes shorter because the refractive index of glass is higher than that of air. If the two beams enter the glass plates in the same phase of oscillation, they will leave it, and re-enter the air, still in parallel oscillations (A_1 and B_1). A third glass plate (Gc) of the same thickness, but darker and, therefore, absorbing a considerable amount of light, changes the amplitude c of a third lightbeam C into c_1 , whereas the amplitudes of A and B will not be changed theoretically by the plates Ga and Gb, because these plates absorb no light. The effect of lightbeam A_1 on the eye is the same as that of B_1 , while lightbeam C_1 appears less bright. A fourth beam D, going through a slightly thicker glass plate (Gd) will leave it in another phase of oscillation than the lightbeams A_1 , B_1 , and C_1 as seen in level X; there is a so-called "phase-difference" (PD) present. The same thing happens if light passes through small particles, which have a different index of refraction or a different thickness than the surrounding medium, as for instance in the case of mitochondria in protoplasm.

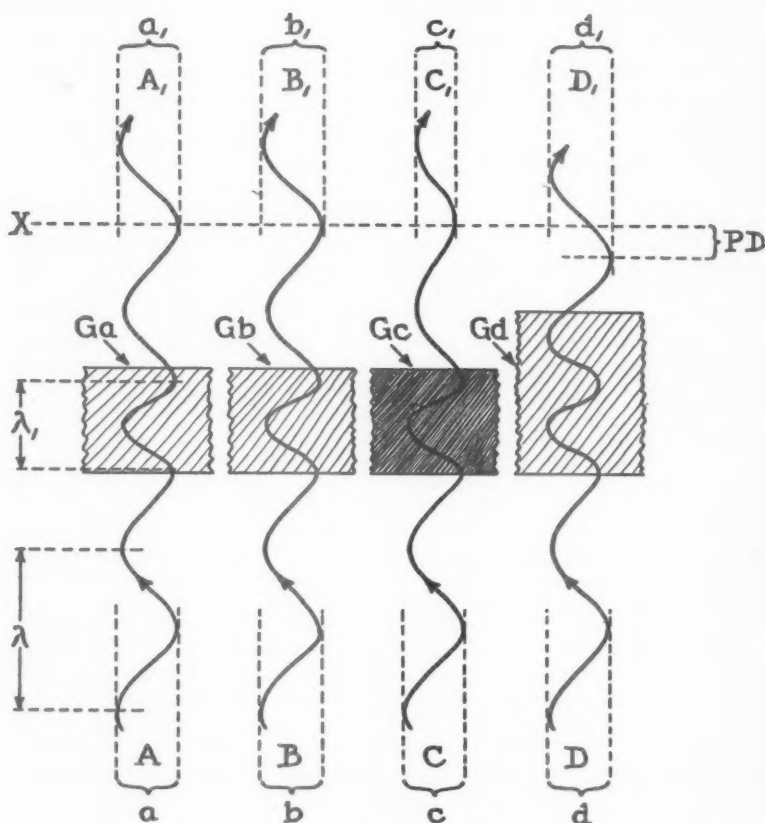
Unfortunately, the eye cannot recognize phase-differences in the ordinary microscope, but only differences in wave length (colors) and in intensity. The PM converts phase-differences into differences in amplitude by means of a "phase plate"; thus, it enables the human eye to perceive phase-differences as black and white contrasts. (For the theoretic explanation of the effect of the "phase plate" see Zernike,¹ Ganz,² and Bennett, Jupnik, Osterberg, and Richards.³) Therefore, the image can be observed with the PM without staining.

In practice, a number of intracellular constituents that are invisible or indistinct when studied by ordinary, darkfield, or ultraviolet microscopy have become readily visible in detail when examined with the

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PM. To provide a comparative illustration of the results obtained with an ordinary and a phase microscope, photographs were made of the cells of an adenocarcinoma of the stomach as viewed in various preparations with the two kinds of optical systems (Figs. 1 to 4). Furthermore, by means of the PM the investigator can follow consecutive intracellular changes for hours and even days in tissue cultures and thus obtain, so to speak, a "longitudinal section" through the whole



Text-Figure 1. Schematic drawing illustrating the effect of different glass plates on light beams which are in the same phase of oscillation. Ga and Gb are made of colorless glass of the same thickness as Gc (dark glass). Gd is made of colorless glass, but it is slightly thicker than the other three plates. This difference in thickness causes a phase-difference (PD) of the beam D.

course of a process, whereas the microscopic section shows but a "transverse section" through the process at the moment when the cell

was killed by fixation. On the other hand, the microscopist should be mindful of the fact that effective phase microscopy depends upon small differences in refraction or thickness between objects. Finally, the fact deserves emphasis that the resolving power of the PM theoretically cannot be higher than that of an ordinary microscope; nevertheless, it is possible to see elements with the PM which are invisible, owing to shrinkage caused by fixation and dehydration, in stained preparations examined with the ordinary microscope.

METHODS

The observations were made with a Zeiss instrument consisting of a centerable condenser with a ring-shaped diaphragm for each objective. The three objectives contained the phase plates. The photographs were taken with a 35 mm. "Alpa-Reflex" camera on Kodak Panatomic X film. As the cells floated freely in fluid, there was always a certain movement under the coverslip; hence photographs were rather difficult to take. Another difficulty was caused by the fact that the cells are three-dimensional. Thus, a certain level of a cell, actually in focus, may still appear indistinct because of the interference caused by elements in other levels.

Suspensions of normal cells of various types (kidney, liver, adrenal, stomach, and small intestine) procured from various animals (frog, rabbit, and mouse) were made by teasing, or washing, or scraping normal organs immediately after the animal was killed. Kidney cells of the frog proved especially suited to study with the PM, because they are not easily influenced by temperature, and they seem to survive the death of the animal for long periods (W. Lewis and McCoy⁴). In order to obtain suspensions of living tumor cells (Gardner's lymphosarcoma, C₃H sarcoma, and granulosa cell tumors of mice, V₂ and Brown-Pearce carcinomas of rabbits), pieces of the neoplastic tissues were removed with aseptic precautions immediately after the animal was killed, thoroughly freed from as much of the adjacent normal tissue as possible, dissected in small pieces, and passed through a 40 mesh Monel-metal sieve.

Physiologic saline (0.9 per cent) and buffered Ringer's solution containing 150 mg. per cent of glucose (BGR) were used as suspension media. Liver cells of the frog required 0.5 per cent of NaCl (Anitschkow⁵), whereas a concentration of 1.25 per cent of NaCl is considered by von Möllendorff⁶ to be physiologic for kidney cells. In order to watch the effects of various chemical agents, the cells were observed while the original suspension medium was replaced in the following

way: A drop of the chemical solution to be tested was placed on one edge of the coverslip and was drawn under the coverslip by means of filter paper placed at the opposite edge of the coverslip, which drew off the excess fluid. Of course, cells also were removed by this procedure, but there still remained a considerable number of cells in the microscopic field adhering either to the coverslip or the slide. During this replacement the cells were constantly observed with the high-power oil-immersion objective; the addition of a small amount of neutral red to the test solution facilitates the determination of the exact moment when the chemical reaches the cells under observation.

Each finding here reported has been confirmed by repeated observations in which different types of cells were used.

OBSERVATIONS

Various distinctive constituents of living unstained cells can be identified readily by means of the PM (Figs. 5 and 7). In undamaged cells, there usually is seen a distinct single-contoured, thin, cellular membrane, no matter whether the cell is isolated (Figs. 5 and 7) or located in the center of a small piece of tissue (Fig. 6). By means of this purely optical method it is impossible to distinguish between the plasma membrane and the extraneous cellular membrane (Chambers⁷).

The Formation of Blisters by Cells of Various Types in Suspensions

Cells in suspensions very often exhibit large "blebs" or "blisters" on their edges (Fig. 8). This phenomenon occurs in almost every kind of cell after the elements have remained in BGR or in isotonic salt solutions of other types for several minutes. In very fresh suspensions of the various cells studied, no blisters could be observed. The only cells that did not form blisters in these experiments were squamous cells from the mouth and tongue of man and frog. In the Shope papilloma only the basal cells showed blister formation.

The first signs of blister formation appear as early as 3 or 4 minutes after the suspension is made: small "cavities" arise in the protoplasm. They are usually adjacent to the cellular membrane, and later, as they grow in size, the cellular membrane bulges out until a blister is formed (Figs. 9 and 10). Then, the blister enlarges chiefly in width, thus detaching the adjacent membrane (Figs. 11 and 12), and finally the whole cytoplasm is surrounded by the contents of the blister, which separate the cytoplasm from the membrane (Fig. 13). Besides this type of blister formation, which may be called "diffuse," there occurs a "local" type (Fig. 14). In this, the base of the blister extends for

only a limited distance around the cell, and, after a time, the blister becomes more rounded. Since these two types can be observed in the same suspension, it does not seem that there is a fundamental difference between them.

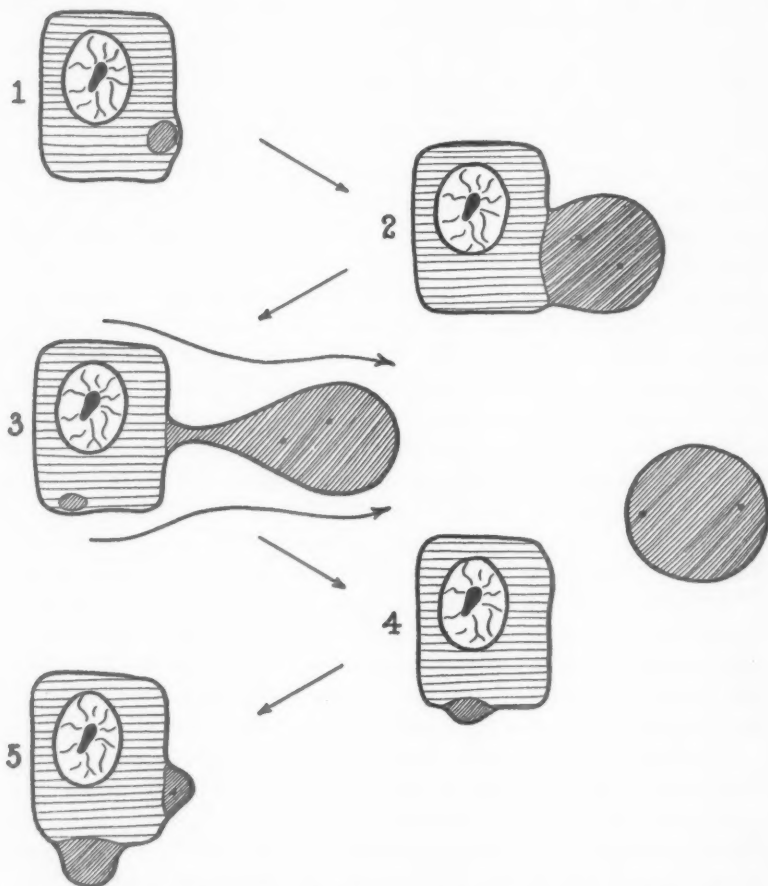
The blister formation in clumps of cells and tissue pieces is usually restricted to the free edges (Fig. 15); in the central region blisters have not been observed. In single cells the blister formation may begin on either side of the cell. Thus, there could never be observed a predilection for the inner face (apical pole) of single gland cells.

Figure 16 shows a very distinct boundary line between blister contents and protoplasm (see also Figs. 11 and 12). Occasionally, minute dark particles can be seen moving about in the blister contents. Their movement continues even if the blister wall is demolished; the suspension medium as a rule contains a number of such freely floating particles. Rarely, the blisters contain one or two round, brilliant, slowly moving granules of considerably larger size which show the same optical structure and the same reactions to chemical agents as do the brilliant granules in the protoplasm (see below). The blisters are filled with a homogenous material, which usually appears slightly darker in the PM than does the suspension medium (Figs. 11 and 12), and is not stained by neutral red in a concentration of 1:10,000.

A distinction can readily be made between blisters and vacuoles (Fig. 17). The latter are found in the protoplasm of "exhausted" cells, that is, cells which have been in the suspension medium for several hours, or have been heated (45° C. for 20 minutes). Vacuoles do not cause bulges in the cellular membrane, and are much brighter than the suspension fluid (Fig. 17). In ciliated epithelial cells, which contain vacuoles, the cilia never move and the nuclei soon disintegrate.

Although two blisters often touch, they usually remain separated by their walls (Fig. 15). This is also true for blisters which are exposed to pressure from one side. Figure 18 shows a cell presenting two blisters which have been moved slightly out of their original position by a fast current in the suspension medium. If the current becomes still more vigorous, the blisters are detached suddenly, and float freely in the suspension fluid. Text-Figure 2 shows schematically the entire process of blister detachment. Just before detachment, the blister becomes drop-shaped and the stalk appears more and more elongated until finally it is torn off. As soon as the blister is detached, it becomes spherical (Text-Fig. 2 and Fig. 19) and behaves like a rubber ball, its surface being momentarily indented if it bounces against an obstacle or a cell in the moving medium, immediately afterwards becoming

spherical again. Strong pressure on the coverslip divides free blisters into multiple small blisters. In detached blisters the black particles enlarge slowly, whereas the occasional brilliant granules do not change their size.



Text-Figure 2. Schematic drawing demonstrating the formation and the detachment of a blister. (1) Blister formation starts in the form of a submembraneous round space in the protoplasm. (2) A big blister with two granules has developed. (3) A strong current of the suspension medium (arrows) stretches the blister which is now drop-shaped. (4) The blister is detached; no defect of the membrane is visible. (5) Formation of a new blister starts again.

It is interesting to note that the cellular part of the blister stalk becomes flattened out immediately after the blister is detached, and a new blister is formed at the same place where the original one was

located (Fig. 20). Under these circumstances, neither the formation of a hole in the cellular membrane nor the outflow of the protoplasm has been observed. The site of the origin of the detached blister becomes completely invisible. Occasionally, new blisters are formed in the base of an already existing blister. These newly formed daughter-blisters grow into the old blister (Fig. 21), and some of them even may develop in the wall of the mother-blisters, especially under the influence of certain chemicals (see below).

Experimental Alterations in the Process of Blister Formation

In order to determine the influence of the suspension medium on blister formation, suspensions of Brown-Pearce and V2 carcinoma cells were studied in normal rabbit serum and rabbit plasma as well as in BGR and isotonic salt solution. The same was done with frog cells of various kinds in frog serum, and the artificial mediums mentioned above. Blisters developed in all of these tests, although somewhat faster in the cells suspended in physiologic saline solution than in those in serum and plasma. There was no other difference in the process of blister formation in the various suspensions.

The cellular membrane disappears in cells suspended in 0.1 to 0.5 M ammonia. If, at the commencement of the experiment, blisters are present (Fig. 22), they are filled up by the swollen protoplasm and the nucleus as soon as 0.05 M ammonia reaches the cells, but the membrane remains visible (Fig. 23). Later, new blister formation may start in such a cell. The cellular membrane is not dissolved by 0.05 M ammonia, but swells, and sometimes daughter-blisters are formed within the membrane itself (Fig. 24). The formation of blisters is relatively independent of the pH of the medium (pH 2.3 to 10.0), but in a medium of pH 10.2 and higher, the membrane disappears and the contents of the blister flow out.

The cellular membrane very often becomes indistinctly outlined in dilute acetic acid, and the slightest pressure on the coverslip is sufficient to destroy the membrane. This change is irreversible. The ciliated epithelial cells show a particular form of reaction to acetic acid (Fig. 25): the cilia, instead of being straight and parallel, become irregular in form and arrangement. The basal bodies appear very dark, and occasionally the entire row of the basal bodies is separated from the rest of the protoplasm by a bright halo (Fig. 25). Therefore, the bases of the cilia behave like an independent section of the cellular membrane which does not shrink as much as does the rest of the membrane.

The formation of new blisters and their further growth is greatly

accelerated by distilled water (Figs. 19, 26, and 27). At the same moment that the distilled water reaches the cells under the coverslip, the nucleus and, to a lesser degree, the protoplasm swell so much that the whole blister is filled up in a few seconds. An instant later, the formation of new blisters starts. The black granules in such blisters swell considerably, particularly in detached blisters (Figs. 19 and 26). By this method it is possible to produce blisters even in cells in mitosis (Fig. 28), although they rarely develop in such cells in physiologic saline solution or in BGR.

Hypertonic salt solution, 0.9 per cent saline of pH 4.0, 70 and 95 per cent alcohol, and acetone, cause a very rapid shrinkage of the blisters, which goes on until the blisters have disappeared and the blister wall lies close to the protoplasm. In the course of this shrinkage, the blister wall becomes slightly wrinkled. In hypertonic salt solution, new blisters appear several seconds later; they grow very rapidly for several seconds and are morphologically indistinguishable from blisters in fresh cells. In the other media mentioned above, no further blister formation occurs, and even the replacement of the chemicals by physiologic saline solution does not produce a detachment of the cellular membrane.

The temperature of the suspension does not play a rôle in the process of blister formation: the amount and the size of the blisters are the same whether suspensions of the same mammalian cells are kept in the ice box, or at 37° or 41° C.

In cells which die spontaneously in the suspension, and in cells killed by heat or by chemicals, blister formation never occurs. Blisters, which are already present before the cells die, do not disappear, but they show no further growth. They remain visible until the cells disintegrate.

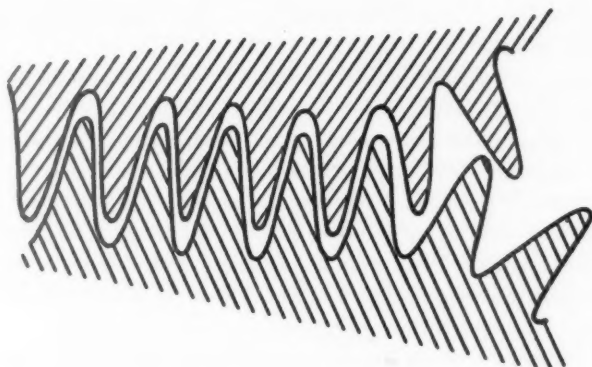
The Extrusion of Blisters from Renal Epithelium

Suspensions of kidney cells occasionally may contain some intact tubules held together by the basement membrane, in which case blisters develop only on the interior surface of the epithelium. The blisters grow very rapidly, and old blisters are very soon detached by the formation of new ones. Thus, free blisters are continuously expelled through the open ends of the tubules (Fig. 29). The same process was observed in unstained frozen sections of living kidney tissue, prepared from a human kidney removed surgically. The unfixed tissue was immediately put into physiologic saline solution and sectioned by means of a freezing microtome with a special knife-cooling device. The

sections were mounted in physiologic saline solution, and the edges of the coverslip sealed with vaseline. Slight, but distinct blister formation was visible in some of the tubules after a few seconds (Fig. 30).

The Cellular Membrane of Squamous Epithelium

Squamous cells were found to have a particular kind of cellular membrane. Suspensions of squamous cells were obtained by scraping the surface of the investigator's oral epithelium with the edge of a coverslip (Bosshard⁸ and von Albertini⁹). The membrane of these



Text-Figure 3. Schematic drawing of the interdigitating wrinkles of the surface of two squamous epithelial cells. On the right is shown the artificial detachment of the two cells.

cells was rather thick and very stiff, thus maintaining the irregular shape of the cells. The membrane was dissolved by 0.5 M ammonia after 1 or 2 hours, whereas the other chemicals mentioned above did not cause any alteration of the membrane. The surface of these cells is delicately wrinkled (von Albertini) and its pattern resembles a fingerprint (Fig. 32), whereas the contour is serrated (Fig. 31). The height of the wrinkles is about 0.5μ , their width 0.1 or 0.2μ . The distance between two wrinkles is approximately 0.3μ . As far as I could see, these wrinkles are folds of the superficial layer of the cellular membrane, interdigitating with those of the adjacent cells (Text-Fig. 3).

DISCUSSION

Meltzer,¹⁰ on theoretical grounds, introduced the expression "pocytosis" for the drinking or sipping of submicroscopic quantities of

water by cells. Later, the term "pinocytosis" was used to describe the intake of whole drops of fluid by "ruffle cellular pseudopodia" in tissue cultures (W. Lewis¹¹). The word "potocytosis" seems preferable for the process of blister formation described in the present paper since an intake of entire drops of fluid by pseudopodia was not observed.

The observations described in this paper demonstrate that potocytosis is a very common process in cells in suspensions. It occurs in epithelial as well as in mesenchymal cells and in normal cells as well as in tumor cells. Squamous cells were the only type which did not show potocytosis. Even distilled water, and 0.05 M ammonia, which increase blister formation greatly in other cells, did not bring about potocytosis in squamous cells.

A process very similar to blister formation was described by Hogue¹² in cells of tissue cultures that were exposed to hypertonic salt solution. However, sometimes blisters developed even in normal Locke-Lewis solution. The detachment of blisters also was observed in Hogue's experiments. Margaret Lewis¹³ described the formation of "blebs" along the edges of tissue cultures exposed to alkali. Similar "blebs" or sacs containing moving granules were observed by the same author in dying cells. These findings indicate that blister formation is not restricted to cells in suspensions.

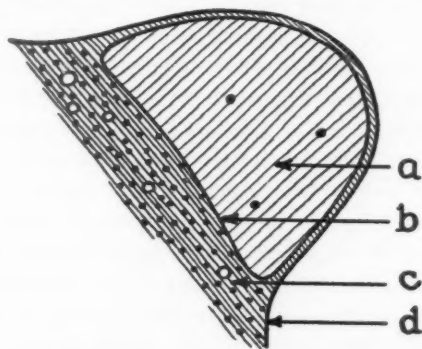
Since blisters fail to develop in cells which are completely surrounded by other cells in tissues, potocytosis must be considered a special type of fluid intake by living cells, which occurs particularly under unnatural conditions, *i.e.*, in suspensions and on the edges of tissue cultures. Therefore, it would appear that one of the fundamental conditions for potocytosis is the presence of a free cell surface in direct contact with an excess of fluid.

Furthermore, the formation and the enlargement of blisters are dependent on a second factor—the viability of the cells—for the enlargement and the new formation of blisters stop immediately when the cells die. This fact indicates that blister formation is an active function of the living cell and even a very low osmotic pressure of the medium is not sufficient to produce blisters in dead cells.

There is only a quantitative difference in the process of blister formation in cells in physiologic saline and those in hypertonic saline solution, distilled water, or homologous and heterologous serum. Hence the chemical constitution of the suspension medium seems not to be of fundamental importance for the process of potocytosis. The acceleration of blister formation in distilled water probably is a consequence of the greater osmotic pressure of the protoplasm as compared with

that of the medium. Under inverse circumstances, the cells being in molar NaCl, the blisters disappear due to the negative osmotic pressure. The subsequent rapid new formation and enlargement of blisters, which at first seem to contradict this explanation, are probably due to an increase in the permeability of the cellular membrane caused by a change in it. The process of blister formation was independent of the temperature of the suspension between 8° and 45° C.

The contents of the blisters seem to be part of the cellular protoplasm in sol-form, whereas the rest of the protoplasm consists of the different granules embedded in the ground substance, which here is a colloid in polyphasic form. Therefore, the granules originally located in the jelled cortex of the protoplasm (Chambers⁷), which is dissolved during the course of potocytosis (gel→sol), are the only ones moving freely in the blister contents later on. This fact proves that the distinct line of separation between the blister contents and the rest of the protoplasm (Fig. 16) is not a proper solid membrane, but a newly formed interface membrane. Therefore, the blister must be considered to be part of the protoplasm, and the blister contents to be outlined by an interface membrane (Text-Fig. 4). The free part of the blister wall behaves optically, as well as in its reaction to chemicals, like the



Text-Figure 4. Schematic drawing illustrating the structure of a cellular blister. (a) Blister contents with a few granules. (b) Internal interface membrane. (c) Protoplasm of the cell. (d) External interface membrane of the blister (plasma membrane).

cellular membrane in blister-free cells. It must be assumed that in regard to their physical and chemical structure these two membranes are identical.

The ability of living cells to form blisters is very interesting in regard to the problem of glandular secretion, for, although the conditions in the experiments reported above are unnatural, it is conceivable that

the secretion of at least some glands takes place in a manner very similar to potocytosis. A process resembling the formation and the detachment of blisters in suspensions has been observed by Jackson¹⁴ in cells of renal tubules of rats which had been fed a very high-protein diet. This author considered blister formation *in vivo* to be a sign of cell injury. The process is not restricted to kidney cells, since Jackson also saw it in uterine glands during pregnancy. Furthermore, the formation of blisters in renal tubules as observed in frozen sections of living tissue is similar to the process of "granuloid formation" in renal tubules (Kosugi¹⁵). Bell's assumption¹⁶ that the "granuloid" is an artifact of extracellular origin is disproved by the above-mentioned observations (see Fig. 30). Although we know the blisters to be a product of living cells, it is not possible at the present time to decide whether potocytosis in kidney cells is restricted to cells surviving the death of the individual or whether it occurs, either under physiologic or under pathologic conditions, while the individual as a whole is alive.

The phenomenon of potocytosis and the reaction of the cellular membrane to various chemicals aid in forming conclusions concerning the nature of the cellular membrane. NaOH (pH 10.2), and 0.1 to 0.5 M ammonia dissolve the cellular membrane, whereas in alcohol, acetone, and, to a lesser degree, in formalin, and in potassium bichromate, the cellular membrane shrinks, probably due to precipitation. Since the protoplasmic ground substance shows the same reactions to these chemicals, the chemical structure of the cellular membrane is likely to be identical or at least very similar to that of the protoplasm.

From the observations presented above, it appears that the membrane of the cells in internal organs is a thin film of a slightly sticky fluid rather than a solid membrane. Otherwise, it would be impossible to explain the fact that a defect caused by the detachment of blisters is immediately closed. A process similar to the detachment of blisters already has been described by Chambers¹⁷: Fat droplets may pass through the cellular membrane (plasma membrane) after intraprotoplasmic injection without causing a visible defect in the membrane. Danielli,¹⁸ using an oil-water interface model, was able to demonstrate the same phenomenon, thus assuming the cellular membrane to be merely an interface membrane. The cellular membrane does not behave like a semipermeable membrane either; the black and the brilliant granules are influenced and changed by hypertonic as well as by hypotonic salt solutions (Fig. 19), thus proving that the membrane is permeable to salts.

The phenomenon of "pinocytosis" (W. Lewis¹¹) is another proof that the cellular membrane cannot be solid. Therefore, the plasma membrane, which represents the only cover of the majority of the cells of the inner organs and of many tumors, has to be considered a simple interface membrane between the cytoplasm and the surrounding medium. The observation that blisters usually do not merge may be interpreted as a consequence of their surface tension. The same force prevents the blisters from rupturing.

The stiff membrane of the squamous cells seems to be identical with the "extraneous cellular integument," Chamber's "proper cellular membrane,"⁷ although I was not able to distinguish it optically from the hypothetic underlying plasma membrane. The observation that these cells fail to develop blisters suggests that the lack of an extraneous cellular integument is a third conditional factor for the formation of blisters.

SUMMARY AND CONCLUSIONS

Potocytosis, the process whereby visible blisters form on tissue cells suspended in liquid media, has been described in detail. Squamous cells alone amongst those studied did not exhibit blister formation, whereas this was almost invariably seen in normal and neoplastic cells of other types when one or more of their surfaces had remained in contact with an excess of fluid for a few minutes. The process of blister formation was accelerated in distilled water and in hypertonic solutions. The blister contents seemed to consist of highly diluted protoplasmic ground substance which was separated from the rest of the protoplasm by an interface membrane. When blisters were detached, the cellular membrane remained apparently intact. It is conceivable that some glandular cells pour out their secretions by the detachment of blisters, and that the "granuloids" seen in renal tubules by Kosugi¹⁵ and others may be detached blisters.

The findings as a whole seem to support the theory advanced by others that many of the cells of the internal organs and those of some tumors are outlined by an interface membrane that lies between the protoplasm and the surrounding medium. Furthermore, the permeability of the cellular membrane to hypotonic and hypertonic saline solutions, as indicated by visible changes in the cytoplasm of cells suspended therein, seems to be much greater than is commonly supposed. The integument of squamous cells, by contrast, seems to be a genuine stiff membrane which is folded into minute wrinkles that interdigitate with those of adjacent cells.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 100

FIGS. 1 to 4 show various preparations of human tumor material (adenocarcinoma of the stomach, biopsy) as seen with the ordinary and the phase microscope (PM). $\times 700$.

FIG. 1. Dry smear stained with Giemsa stain; ordinary microscope. The nuclear and protoplasmic elements are very indistinct and the cells are markedly shrunken.

FIG. 2. The edge of an unstained frozen section of fixed material; PM. The nuclear constituents are readily visible, whereas the protoplasm, due to the formalin effect, is granular.

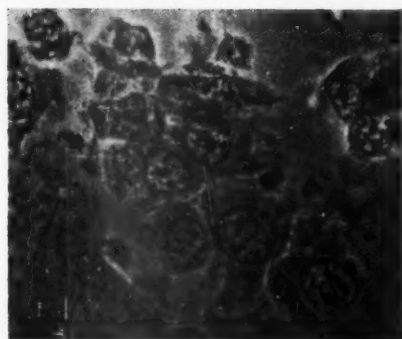
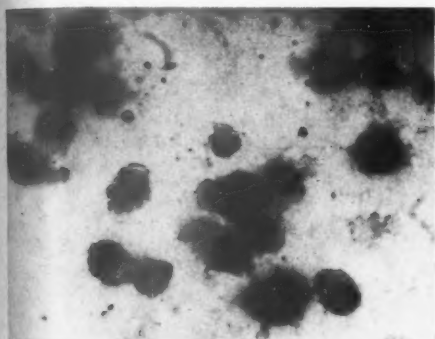
FIG. 3. Paraffin section of Zenker-fixed material; hematoxylin and eosin. The cells have shrunk and the nuclei are irregularly outlined. The nuclear elements are less distinct than in Figure 2; the protoplasmic structure is about the same. (Ordinary microscope.)

FIG. 4. Unstained cells in suspension; PM. The cells are not shrunken, the elements of the nuclei and the protoplasm being readily visible. *a* demonstrates a giant nucleus, *b* shows a multinucleated cell, and *c* illustrates a mitotic cell (metaphase).

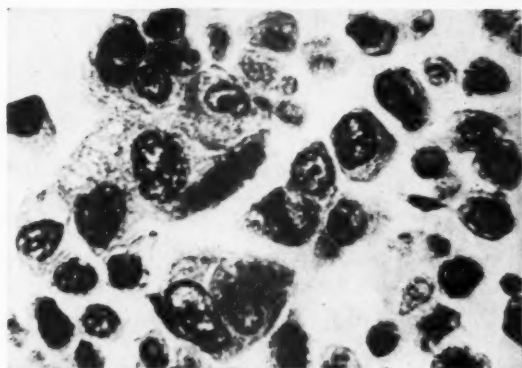
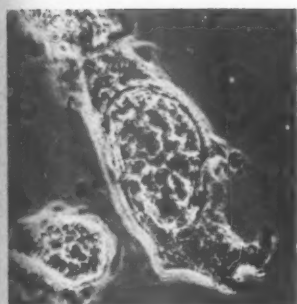
FIG. 5. Fresh suspension of frog kidney cells. The elements are distinctly outlined by the cellular membranes. The protoplasm is filled with small, dark granules and the nuclei are "clear," containing a few dark dots. PM. $\times 700$.

FIG. 6. Piece of a renal tubule of a frog. The cellular membranes are easily seen, while numerous small, black granules are visible in the cytoplasm of all of the cells. In the peripheral cells they are round and enlarged, while in the central area they are more rod-like. PM. $\times 700$.

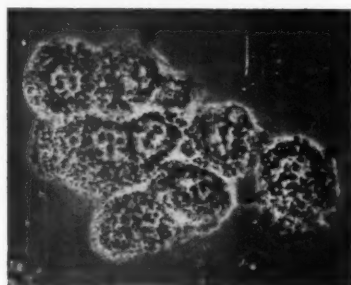
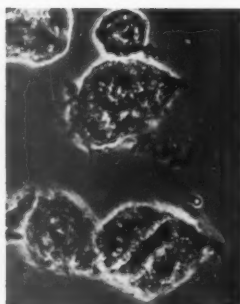
FIG. 7. Cylindrical cell of the stomach, surgical specimen. There are three types of protoplasmic granules visible in the homogenous ground substance: (1) five large, white, spherical droplets on the left of the nucleus; (2) a group of very small, black granules in the apical pole (on the left) and near the base of the cell (on the right); and (3) many dull-gray granules in the basal part. PM. $\times 1400$.



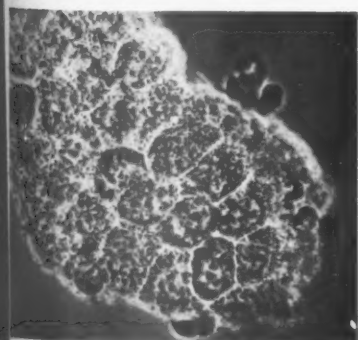
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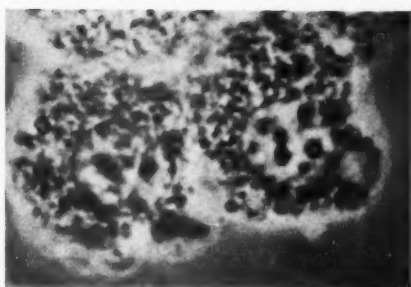
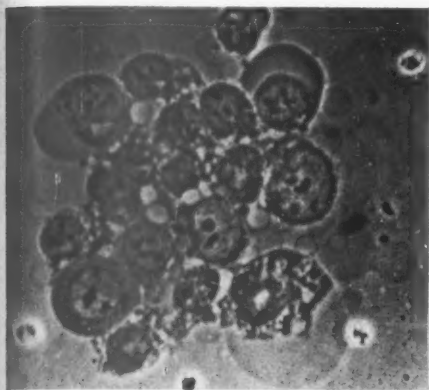
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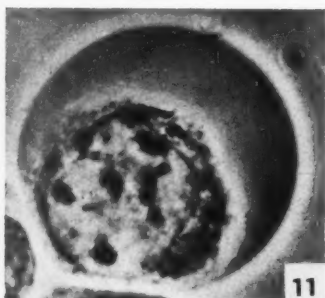
Phase Microscopy, Potocytosis

PLATE 101

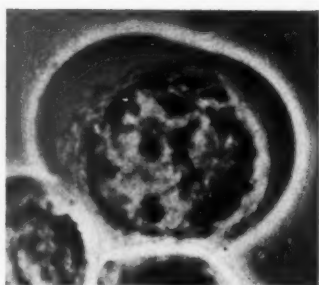
- FIG. 8. Brown-Pearce carcinoma cells, after having been in the suspension under the coverslip for 30 minutes, showing extensive blister formation. In the right middle cell there are several small blisters in the contents of a large blister. Above this cell there is a free blister. The process of detachment of a blister can be seen in its terminal phase below the cell just mentioned. PM. $\times 700$.
- FIG. 9. Intraprotoplasmic origin of a blister (on the right) in a kidney cell of the frog, 4 minutes after the animal was killed and the suspension was made. PM. $\times 1400$.
- FIG. 10. Brown-Pearce carcinoma cell containing one intraplastic and one bulging blister. PM. $\times 1400$.
- FIG. 11. V2 carcinoma cell showing diffuse blister formation. The protoplasm is compressed by the blister contents; the latter appear slightly darker than the suspension medium. The blister is surrounded by a bright diffraction ring. PM. $\times 1400$.
- FIG. 12. The same cell as shown in Figure 11, 10 minutes later. The blister is markedly enlarged, and a second bright ring between the protoplasm and the blister contents has developed. PM. $\times 1400$.
- FIG. 13. Granulosa cell tumor showing marked diffuse blister formation after the suspension was in the ice box for 24 hours. The cell in the center, besides being "ballooned" diffusely, contains a spherical vacuole, the contents of which are much brighter than that of the blisters. PM. $\times 1400$.
- FIG. 14. Rabbit sarcoma cell exhibiting a single "local" blister. Of note is the distinct line between the blister contents and the rest of the protoplasm. PM. $\times 1400$.
- FIG. 15. Blister formation on the edge of a cortical piece of a frog kidney, 2 hours after the suspension was made. Some of the blisters are detached and stick on the surface of other blisters, but they do not merge. Several small granules are visible in some of the blisters. PM. $\times 850$.
- FIG. 16. Large blister in a kidney cell (frog) containing some relatively large, black granules (out of focus). The blister contents are separated from the compact protoplasm by a distinct line. PM. $\times 1400$.



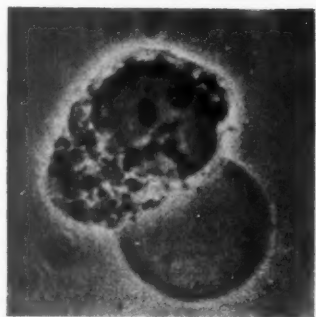
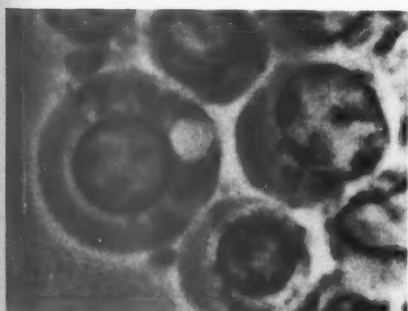
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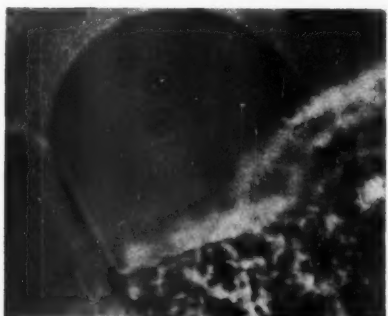
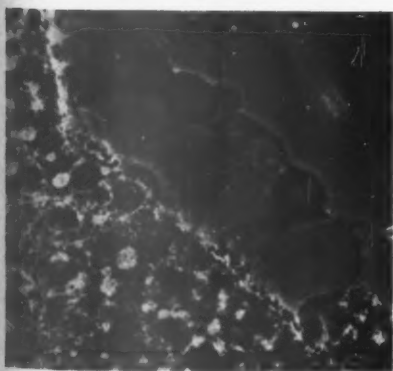
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Phase Microscopy, Potocytosis

PLATE 102

- FIG. 17. Degenerated Brown-Pearce carcinoma cell after having been in the suspension at 37° C. for 5 hours. There are numerous small, bright vacuoles in the protoplasm. PM. $\times 1400$.
- FIG. 18. Brown-Pearce carcinoma cell. An artificial current of the suspension medium under the coverslip from the left to the right deflects two blisters, but they do not merge. PM. $\times 1400$.
- FIG. 19. Artificial blister of a ciliated frog cell in distilled water. On the left is a detached blister, containing some enlarged dark granules. PM. $\times 1400$.
- FIG. 20. Kidney cells of the frog. Formation of a new blister may be seen in the cell on the left after a blister has been detached previously. The new blister again contains some black granules. PM. $\times 1400$.
- FIG. 21. Brown-Pearce carcinoma cell showing numerous blisters, two of which have developed within the contents of a large blister. PM. $\times 1400$.
- FIG. 22. Ciliated cells of the frog pharynx. The chromatin network is out of focus; large spontaneous blisters have developed. PM. $\times 1400$.
- FIG. 23. The same cell as shown in Figure 22, 3 minutes after ammonia has been added to the suspension. The cilia have begun to disintegrate, the small, black, intraprotoplasmic granules (see Fig. 22) are very indistinct. The nucleus is enlarged and its membrane has almost disappeared. PM. $\times 1400$.
- FIG. 24. Frog kidney cell in 0.05 M ammonia, showing a large blister. The blister was already present before the ammonia was added to the suspension, but the ammonia caused a marked enlargement of the nucleus, which now fills the whole blister. A small new blister has been formed within the blister wall. PM. $\times 1400$.
- FIG. 25. The effect of 5 per cent acetic acid on a ciliated cell of the frog pharynx. The cilia are curled and their basal bodies are detached as a whole from the rest of the protoplasm. The nuclear membrane is double contoured and brilliant. PM. $\times 1400$.

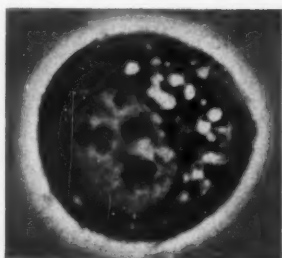
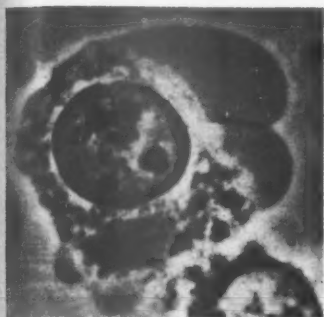
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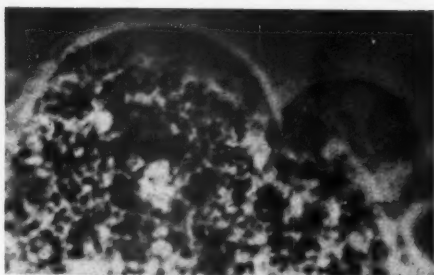
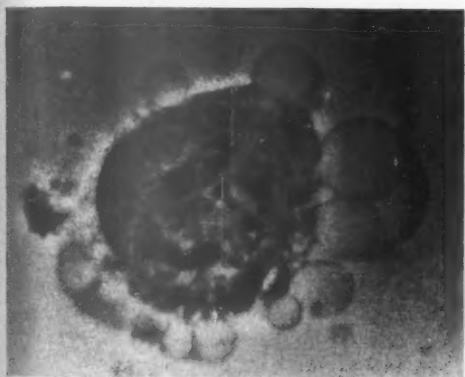
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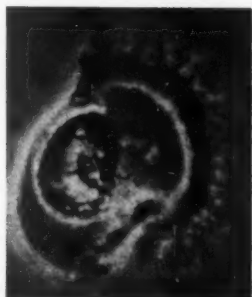
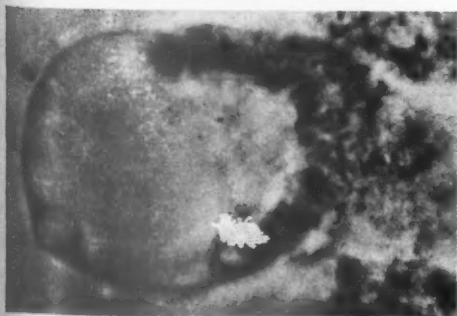
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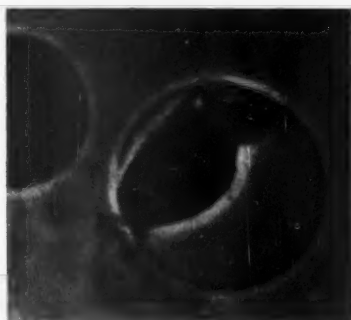
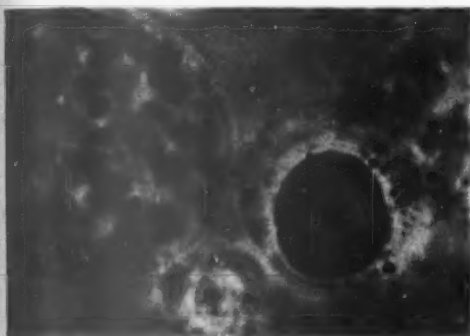
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Phase Microscopy, Potocytosis

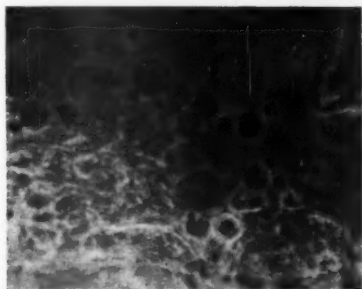
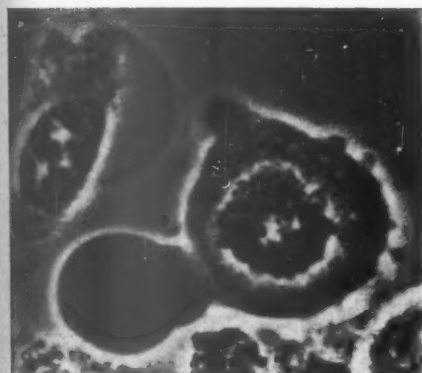
PLATE 103

- FIG. 26. Brown-Pearce carcinoma cells with artificial blisters, caused by distilled water. The entire cellular membrane, stretched out by a high intracellular tension, seems to form the blister wall. The nucleus on the right is hazy and homogenous, its nucleolus as well as the nucleus of the cell on the left are out of focus. The protoplasmic granules are enlarged. PM. $\times 1400$.
- FIG. 27. Artificial old blister of a C₃H sarcoma cell, which has been in distilled water for one hour. There are some small vacuoles in the compressed protoplasm. PM. $\times 1400$.
- FIG. 28. Spontaneous blister formation in C₃H sarcoma cells. The cell on the right is in mitosis and its blister shows stalk formation. A bright halo surrounds the chromosomes, and small, black particles are present in the protoplasm of this cell. PM. $\times 1400$.
- FIG. 29. An intact tubule of a mouse kidney has poured numerous blisters out of its open end. PM. $\times 700$.
- FIG. 30. Unstained and unfixed frozen section of the cortex of a human kidney in physiologic saline solution. The section was made immediately after the surgical removal of the organ: the cells are still living and show blister formation into the lumen of a tubule. PM. $\times 400$.
- FIG. 31. Cells of the same cell type as shown in Figure 32, in profile. The wrinkles are rather high and very sharp; the protoplasm contains many irregular, dark elements. PM. $\times 1400$.
- FIG. 32. Upper surface of a squamous cell of the human mouth. The delicate wrinkles are clearly visible, as are the sharp edges of the cell. Some bacilli stick on the cellular surface. PM. $\times 1400$.

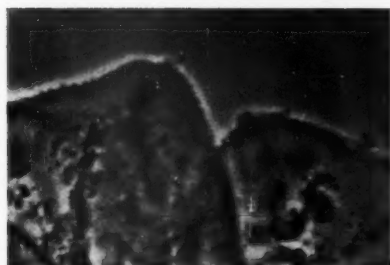




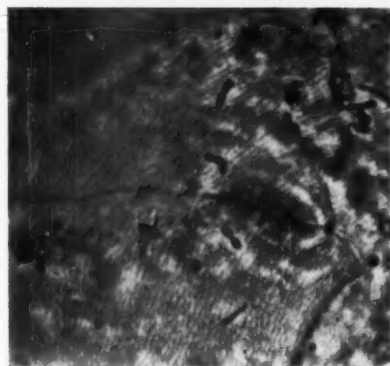
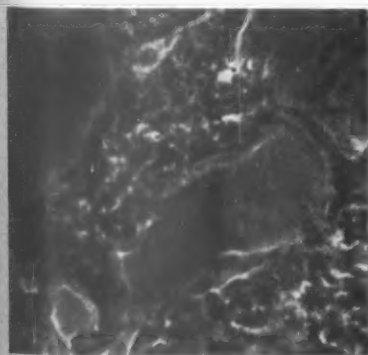
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Zollinger

Phase Microscopy, Potocytosis

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CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE

II. THE MITOCHONDRIA AND OTHER CYTOPLASMIC CONSTITUENTS UNDER VARIOUS EXPERIMENTAL CONDITIONS *

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It has long been known that formed bodies of various sorts are present in the cytoplasm of tissue cells, those called mitochondria or chondriosomes having especially attracted the enduring interest of cytologists. The aims of the studies herein reported have been to identify the constituents of the protoplasm that can be seen with the phase microscope, and to learn more about their structure and chemical composition.

OBSERVATIONS

The working principle of the phase microscope (PM), the technic, and the material used in the experiments were described in the preceding paper.¹ By means of the PM three elements have been seen in the protoplasm of a variety of nucleated cells as follows: (1) a gray, mostly homogenous ground substance; (2) numerous small, dull-gray or black granules; (3) a few brilliant bluish granules of various sizes and shapes.

Ground Substance

Using the highest possible optical enlargement ($\times 1400$), very small, barely visible granules sometimes are observed in the homogenous ground substance, but the protoplasm of living cells never displays a network structure. In dead or dying cells, however, the protoplasm appears as a very dense network of irregular, thin fibrils, too small to be photographed, although readily recognizable in fixed and stained cells.

Mitochondria

The black or dull gray granules seen in the protoplasm of cells apparently are mitochondria. When viewed with an ordinary microscope these same granules appear pale yellow, and are stained with Janus green.

When very fresh cells of the kidney and the small intestine are viewed with the PM, these granules are rod-shaped and about 0.1μ wide and from 0.8 to 1.2μ long. In kidney cells these granules, or

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mitochondria as they will be called henceforth, very frequently show a small bulb on one end. In cylindric cells, such as those of the intestine, the mitochondria usually are located in the basal parts of the cells. If there are rod-like mitochondria, they are often oriented vertically to the basal membrane of the renal tubules or to that of the intestinal glands. In liver cells the mitochondria are arranged around the nucleus in a radial manner.

When cells have been in the suspension medium for a few minutes, the mitochondria usually become round, with a diameter of from 0.25 to 0.7 μ . The longer the cells remain in the medium the larger and more spherical become the mitochondria. Low temperature (below 37° C.) delays the change.

The size of the mitochondria varies not only from cell to cell of the same type, but even from one mitochondrion to another, this variation occurring in normal as well as in tumor cells. There is no difference in size or form between the mitochondria of the kidney cells of well fed frogs and those in cells of frogs which are starving. Rod-like mitochondria seem to be rare in tumor cells.

The mitochondria usually move slowly to and fro in the protoplasm. The looser the protoplasm, the faster is this movement, and the larger the amplitude. Since the mitochondria of destroyed cells, floating freely in the medium, show the same movement, it is probably due to the brownian movement of the surrounding molecules. These free mitochondria disintegrate only after several hours at 37° C. Intermediate stages between fat droplets (see below) and mitochondria cannot be seen.

Figures 3 to 5 demonstrate the cellular reaction to distilled water. The cells swell immediately and become spherical. The mitochondria enlarge greatly (Fig. 2) and sometimes appear as small vesicles. This enlargement is particularly striking in mitochondria of destroyed cells floating freely in the suspension medium. The thin walls of the vesicles look like membranes, each showing one distinct black, regular thickening (Fig. 7), which can be observed only in profile. Since the mitochondria are always rolling in the moving suspension medium under the coverslip, it can be observed that there is such a thickening in every vesicular mitochondrion. The material forming the thickening seems to be located on the internal side of the wall of the vesicle in every case, and projects into the interior of the vesicles. Therefore, the outline of these vesicular mitochondria is always smooth. These changes are reversible, and the mitochondria decrease in size when physiologic saline solution replaces the distilled water, and eventually reach their

original size (Fig. 8). The whole process can be repeated several times, the result always being the same.

Occasionally, blisters¹ contain mitochondria which swell immediately when reached by the distilled water under the coverslip, even before the swelling of the intraprotoplasmic mitochondria (Fig. 9).

In 0.05 to 0.1 M ammonia the mitochondria enlarge greatly (maximal diameter, $3.2\ \mu$), and take the shape of spherical balls, or even vesicles, with slightly irregular walls. The distinct regular thickening observed in distilled water does not appear. This enlargement in ammonia is especially striking in those mitochondria that float freely in the suspension medium (Fig. 11). After a minute or two in ammonia, some of these swollen mitochondria merge and form spherical, black balls, the diameter of which ranges from one-tenth to one-fifth of that of the swollen nucleus. The replacement of the ammonia by physiologic saline solution brings about a slow shrinkage of the single mitochondrion, whereas the coalesced mitochondria do not change their size (Fig. 12). In 0.5 M ammonia the whole cell is transformed into a jelly, the slightest pressure on the coverslip causing disintegration of the cell, after which the mitochondria float freely in the suspension medium as a compact mass.

Ten per cent formalin causes the whole cell to shrink slightly, and the mitochondria become very small but do not change their shape. The shrinkage of the protoplasm is much more striking in 100 per cent formalin; the cells become irregular, round, and yellow. These changes are irreversible. Seventy and 95 per cent alcohol, acetone, and, to a lesser degree, 3 per cent potassium bichromate have the same effect. In 5 per cent acetic acid there is no recognizable shrinkage of the protoplasm, but the mitochondria swell markedly; the replacement of this agent by physiologic saline solution does not yield any further change. Two surface active compounds investigated (0.1 per cent hexylresorcinol S.T. 37,* and zephiran†) cause a marked swelling of the protoplasm; in hexylresorcinol the mitochondria shrink irreversibly, whereas they remain unchanged in zephiran.

In order to determine the effect of various pH concentrations on living cells, the pH of 0.9 per cent NaCl was changed by means of NaOH and HCl, respectively, and the pH was measured with a glass electrode. In a medium of pH 5.8 to 4.0, the mitochondria enlarge slightly and reversibly (Fig. 10); the same is true at pH 8.3. At a lower H-ion concentration (10.1) the mitochondria swell more than at 8.3, and about 2 minutes later the whole cell begins to disintegrate. The en-

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largement of the mitochondria in alkaline media is reversible if the cells subsequently are washed in buffered physiologic saline solution of pH 7.0.

At 37° C. the mitochondria enlarge progressively until they reach their maximal size after about 2 hours. Afterwards they rapidly decrease in size. At 45° C. the maximal diameter is reached after 5 to 10 minutes, and at 56° C. after only 2 minutes. At 75° C. the mitochondria do not enlarge. Suspensions which have been in the ice box show very small mitochondria, but if the suspension is placed in the water bath at 37° C., the mitochondria enlarge much faster than do those of the fresh suspension.

Storage Granules

In fresh suspensions of normal organs the protoplasm very often contains several small, brilliant granules, which may be called "storage granules" (see discussion for explanation). These storage granules enlarge considerably when the suspension is kept at 37° C. for several hours. The enlargement is accelerated by high temperature (not exceeding 45° C.), and delayed by low temperature (Fig. 13). These granules are bluish under the PM and yellow-green under the ordinary microscope, and they show a very distinct, black contour (Figs. 1 and 2). Usually, the storage granules are spherical, but sometimes their shape is slightly irregular. Their diameter ranges from 0.1 to 2.0 μ . In liver and kidney cells, these brilliant granules are large and very numerous; in the stomach and the intestinal epithelium, their size and number are moderate, whereas in the ciliated epithelium and particularly in fibrocytes they are very small and infrequently found. In these latter cells they enlarge only slightly at 37° C. In tumor cells the brilliant granules are less numerous than in normal cells, but sometimes they are very large; usually the nuclei of such cells exhibit signs of degeneration. In most cells the storage granules are scattered throughout the protoplasm, but a particular arrangement of these granules around the intracellular mucus or, if there is no secretion, in the apical pole of the protoplasm is found in the cells of the stomach and the intestinal epithelium.

The storage granules sometimes move to and fro in the protoplasm, but not as much as do the mitochondria. The granules, after destruction of the cells, float freely in a physiologic medium and do not change their size or shape.

In suspensions of living cells, the storage granules are not stained by neutral red in a concentration of 1:10,000, but they stain deep red in cells which show disintegration of the nuclei or if too strong a con-

centration of the dye is used. In the latter case, the nuclei are damaged by the dye. If the cells are crushed by pressure on the coverslip, the storage granules remain almost unchanged (Fig. 14).

Distilled water, 0.05 M ammonia, 4 per cent formalin, 3 per cent potassium bichromate, and 0.1 per cent hexylresorcinol do not alter the storage granules. In 0.1 to 0.5 M ammonia, 5 per cent acetic acid, acidic media of pH 4.0 and higher, acetone, 95 per cent alcohol, and 0.1 per cent zephiran, the granules remain unchanged for some minutes. Then, suddenly, they disappear one after the other. However, there remains a wrinkled, thin, black shell for every granule. This can be demonstrated particularly well in granules floating freely in the medium. Unfortunately, these shell-like remains are too small (0.2 to 0.3 μ) to be shown in photomicrographs. With the exception of 0.1 M ammonia, this partial dissolution is irreversible in all of the experiments. In a solution of 0.1 M ammonia, the shell-like remains of the storage granules swell somewhat when the ammonia is replaced by physiologic saline solution, and they become spherical again, but never brilliant. Occasionally, neighboring storage granules may merge before being partly dissolved; in this intermediate phase they form large, brilliant, spherical globules. Molar saline solution causes a slight decrease in size of the storage granules combined with irregularities in shape; these changes are irreversible.

Granules of a particular kind can be seen in squamous cells of the epithelium of the mouth. Figure 15 demonstrates such a cell showing a pyknotic nucleus and numerous irregular, black granules. In other cells of the same origin the granules are much larger, brilliant yellow, and almost spherical, with some small indentations or sharp edges (Fig. 16). They never show brownian movement. In suspensions of Shope papilloma cells (Fig. 17) and, to a lesser degree, in those of the V2 carcinoma (originating in the Shope papilloma) these granules are very large and numerous. They show the typical staining reactions of keratohyalin (Ladewig and Oberndorfer²).

Microsomes

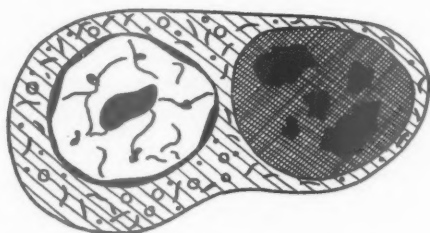
Blisters may contain some mitochondria and even storage granules.¹ In addition, under the influence of distilled water some very small particles appear in the blister contents. These particles move to and fro, and may almost reach the size of mitochondria in normal cells. The replacement of the distilled water by physiologic saline solution produces a sudden shrinkage and disappearance of the particles. These elements also can be observed in the blister contents when 0.05 M

ammonia has been applied, but they are more indistinct than in the experiments with distilled water.

After many failures, success was attained in photographing these minute particles. Apparently, when the particles are exactly in focus, they are too small for photographic reproduction, whereas, when the picture is focused on a plane slightly higher or lower than the particles, they appear as small, indistinct, black spots on the film. The results of making several exposures of the same cell and focusing at different levels are demonstrated in Figures 9 and 18.

Fat Droplets

Some cells, especially those of the liver and kidney, sometimes contain small or large (0.5 to $3.0\ \mu$), brilliant yellow droplets which are absolutely spherical and show a dark double contour (Fig. 19). When



Text-Figure 1. Brown-Pearce carcinoma cell containing a large "inclusion body," which shows large fragments.

the cells have been destroyed, these droplets float freely in the suspension medium. They never change their form and size during the experiments and do not move within the cells. Since the droplets are immediately dissolved by 95 per cent alcohol and selectively stained by scarlet red, there seems little doubt that they are composed of fat.

Inclusions

Cells of the rabbit sarcoma, the Brown-Pearce and V2 carcinomas, as well as those of the C3H sarcoma, often contain large protoplasmic inclusion bodies. They are mostly spherical or oval and bright yellow (Fig. 20). When the suspension contains neutral red, 1:10,000, very often large, bright red granules appear within the inclusion bodies. Occasionally, these inclusion bodies contain several black, irregular particles which measure from 1 to $3\ \mu$ in diameter (Text-Fig. 1). It seems probable that these bodies represent dead cells, the black particles being fragments of their nuclei.

Vacuoles

Sometimes there are large, more or less rounded spaces in the protoplasm of cells which have been in suspension at 37° C. for several hours, or under the coverslip for 20 to 30 minutes (Figs. 21 and 22). They are irregularly outlined, and their contents stain slightly with neutral red. In unstained preparations the contents of vacuoles are much brighter than those of blisters.¹ These vacuoles never cause bulges of the cellular membrane, and do not contain particles. Therefore, the vacuoles are readily distinguishable from blisters.

In the ciliated epithelium of the frog pharynx and in a few tumor cells, round spaces occasionally may be seen located on the poles of the nuclei (Fig. 23). These spaces are less distinct, but even brighter than the vacuoles; it is possible that they represent the Golgi apparatus, but positive proof of this suggestion is not available. On the other hand, pictures like those presented by Brice, Jones, and Smyth,³ showing the Golgi apparatus as a large dark mass, were not observed in the experiments mentioned above.

DISCUSSION

The protoplasmic ground substance, in which the nucleus and the various granules are embedded, is said to consist mainly of a framework of fibrous proteins, as well as nucleoproteins with lipids and a mesh-filling interparticular fluid (Bensley,⁴ Guilliermond⁵). Claude⁶ assumed that the microsomes form the chromophilic part of the ground substance. Under normal conditions, this substance appears homogeneous and slightly gray under the PM; but it becomes net-like when the cells die as a result of heat. The sudden shrinkage and the fact that the protoplasm becomes irreversibly brilliant when placed in acetone, alcohol, etc., indicate that these chemicals cause a precipitation. It is very difficult to decide whether the ground substance is a sol or a gel. Lewis⁷ demonstrated the reversibility of the protoplasmic gelation produced by acid mediums and the dissolution of the protoplasm by alkali; however, in the experiments mentioned above these changes were irreversible. The brownian movement of the various protoplasmic particles, which may be seen in almost every cell, has led Bayliss⁸ to the conclusion that the protoplasm is a sol, whereas Lewis⁷ observed brownian movement only in cells whose protoplasm had been dissolved by alkali. As a matter of fact, the brownian movement differs very much from cell to cell, and even from particle to particle. This indicates that the protoplasm is a colloid in a polyphasic state, of which the proportion of the two phases (gel and sol) may change very easily.

The mitochondria can easily be observed in living cells by means of the PM; they are small, black, dull granules which may or may not show brownian movement. The shape of the mitochondria changes from cell to cell; rod-like mitochondria are rather rare in cells suspended in artificial mediums. The mitochondria react very rapidly to changes of the medium. Their enlargement in hypotonic solutions has already been observed by Anitschkow⁹ and by Bang and Sjövall.¹⁰ The vesicular swelling caused by the action of distilled water probably is a preponderantly osmotic effect; but no chemical constituents of the mitochondria can be observed to be dissolved by distilled water. In every freely floating vesicular mitochondrion there appears in distilled water a peculiar, strictly localized thickening of the wall (Fig. 7). Therefore, this thickening must be an integral constituent of every mitochondrion; it seems to represent the original body of the mitochondrion, the membrane or integument of which has become detached by the action of the distilled water. A comparison between this process and potocytosis¹ is obvious: a localized increase of the intracellular and especially the intraparticular water content takes place. Mirsky and Pollister¹¹ stated that molar NaCl dissolves desoxyribonucleoprotein selectively. The experiments reported above suggest that molar saline solution does not dissolve any constituent of the mitochondria. Provided that Mirsky and Pollister's assumption is correct, this indicates the lack of soluble desoxyribonucleoprotein in the mitochondria. This result, obtained by the direct optical method (PM), corresponds with the former findings of these authors,^{12,13} who used chemical analysis and an indirect optical method (microscopic sections), as well as with those of Caspersson and Santesson¹⁴ (microspectroscopy). Osmosis may partly explain the action of ammonia on the mitochondria, but their coalescence in ammonia indicates that the surface tension of the mitochondria decreases under the influence of ammonia.

The observation of a vesicular structure of the swollen mitochondria harmonizes with those of Guilliermond,⁵ Anitschkow,⁹ Hertwig,¹⁵ Cowdry,¹⁶ and Ludford.¹⁷ These morphologic findings seem to support the statement that the chemical constituents of the mitochondria, the lipid and protein molecules particularly, are concentrated in the superficial zone of each mitochondrion (Bensley,¹⁸ Bourne¹⁹). Observations with the electron microscope (Claude and Fullam²⁰) confirm the existence of a membrane forming the external zone of the mitochondrion. Opie and Lavin,²¹ using Giemsa's stain and ultraviolet light, have proved in recent investigations that the external layer of mitochondria consists of ribonucleic acid. Lewis⁷ and Hogue²² found

such vesicular mitochondria in tissue cultures treated with acids and anisotonic salt solutions, respectively. Both authors considered cells containing vesicular mitochondria to be dead.

Since the shrinkage of the mitochondria in alcohol and acetone is irreversible, it may be assumed that alcohol and acetone dissolve the lipid constituents, and perhaps precipitate the proteins.

The reaction of the mitochondria to heat (45° to 65° C.) indicates that these temperatures first accelerate and then delay a process, which takes place in every cell of a suspension at 37° C., and even at room temperature. The higher the temperature, the sooner it occurs. The visible sign of this process is the enlargement of the mitochondria. The significance of this enlargement is unknown; it might be caused by the change of the intracellular H-ion concentration as a result of pathologic cellular metabolism in an unnatural surrounding.

Chambers²³ succeeded in breaking mitochondria into two pieces with the microneedle; Strangeways and Canti²⁴ and Claude⁶ observed mitochondria spontaneously breaking in two. Therefore, the contents of the mitochondria are not likely to be in a fluid state.

When reviewed in their entirety, these experiments and reflections lead to the following conclusion: the mitochondrion consists of an elastic membrane and its contents which, under ordinary conditions, are probably in gel form. Important components of the mitochondria seem to be located in, or just below, the membrane. When the mitochondrion swells by intake of water (in distilled water, etc.), the membrane is detached from the "body" of the mitochondrion by the fluid.

Finally, the fact deserves emphasis that the mitochondria react rather rapidly to every chemical change of the medium, but are not very fragile; very often they "survive" the mechanical destruction of cells for some time.

The "storage granules" are spherical or slightly irregular, brilliant granules with a bluish color in the PM. They are very resistant to mechanical influences, and thus the suspension medium usually contains a great number of these particles. The number of storage granules is surprisingly high in liver and kidney cells (storage granules: mitochondria = 2:1, or 1:1). The discrepancy between this statement and the findings in microscopic sections, in which von Möllendorff²⁵ described extremely rare granules in kidney cells, can be explained easily by the dissolution of large parts of the storage granules by fixatives and dehydrating chemicals (see below). The differential diagnosis between storage granules and fat droplets as observed with the PM can be made easily by means of alcohol: fat is immediately and

completely dissolved, whereas the storage granules disappear later, leaving shell-like remains.

The reaction of the storage granules to various chemicals (alcohol, acetone, acetic acid, etc.) indicates that these granules consist of at least two different materials. One component, which gives the storage granules their bluish color and their brilliancy in the PM, and the yellow color in the ordinary microscope, is dissolved under the influence of these chemicals. The other component is probably precipitated by these agents, and remains visible in the form of a "shell." Claude,⁶ using differential centrifugation, found that phospholipid and ribonucleic acid were the main constituents of the storage granules of liver cells. Therefore, the storage granules must be considered to consist of a solid membrane and its fluid contents, which are rich in lipids. The solid structure of the membrane is proved by the facts that (1) the storage granules may be irregularly outlined, and (2) a wrinkled "shell" remains after the action of the chemicals mentioned above. The contents of the storage granules must be fluid, or at least not solid, because the granules burst immediately when punctured by a micro-needle (Chambers²³).

Due to the small size of the "shells," the determination of their chemical composition is difficult. The main material is probably protein. Deane,²⁶ in a recent paper on the basophilic bodies in hepatic cells, demonstrated that these bodies consist of ribonucleoprotein. These bodies may be identical to the remains or "shells" of the storage granules. If this is true, the variability of these bodies in size and form (Deane) could be a consequence of the different sizes which the storage granules had reached at the moment of fixation, as well as of the degree of their disintegration caused by fixation and dehydration. In further experiments I shall try to decide whether my assumption is correct.

The storage granules are by no means a homogenous group of granules; without doubt this group includes elements of different functional properties:

(1) Proof of the secretory function of storage granules of one type is offered by Claude,⁶ who observed an accumulation of the "secretory granules" in liver cells of the starving *Amphiuma tridactylum*. After the animal's first meal, these granules were poured out. A cyclic change of the "secretory granules" in gland cells is assumed by Maximow and Bloom,²⁷ and many others. The general conception is that these genuine secretory granules store the secretion products, or their precursors, until they are poured out.

(2) It was stated above that the storage granules observed in these experiments enlarge in living cells in suspension, and that this enlargement depends upon the temperature of the suspension medium as well as upon the length of time which the cells stay in the suspension. In fresh suspensions, on the other hand, particularly in those of malignant tumor cells which have been thoroughly washed in physiologic saline solution and then stored in a protein-free medium, the main building material of the storage granules has to be considered of endocellular origin. It has to be assumed, therefore, that granules of this particular type store accumulated waste products of the cellular metabolism. The same statement was made years ago by Lewis and McCoy,²⁸ who proposed to call these particles "starvation granules," whereas Ludford¹⁷ named them "degeneration granules." *In vitro*, a secretion of such granules never was observed, and their further fate *in vivo* is unknown also. It is conceivable that such cells, as, for instance, in the neighborhood of an infarct, can recover and later remove the granules.

(3) A further subgroup is formed by the "resorption granules," which have been studied particularly by Oliver²⁹ in the cells of renal tubules. He demonstrated the accumulation of large protein granules in cells of certain sections of the nephron after intraperitoneal injection of heterologous protein into animals. These granules in all probability represent heterologous protein, resorbed by the tubular cells from the primary urine after having passed through the glomeruli. These "resorption granules" are a common finding in human glomerulonephrosis (Zollinger³⁰). The fundamental process leading to the formation of resorption granules is the cellular resorption and the intracellular accumulation of a primarily extracellular material.

It has been emphasized that the storage granules are not a homogeneous group; however, the experiments reported above demonstrate that the optical behavior as well as the structure of the three subgroups in the PM are the same. Furthermore, the three subgroups are founded on the intracellular storage of either exogenous or endogenous material, probably containing proteins. For these reasons, the concentration of the three subgroups in one main group, called "storage granules," seems to be justified.

According to these theories, the granules of squamous cells would represent storage granules, which consist in this case of keratohyalin. These brilliant particles appear first in the deepest layers of the multilayered squamous epithelium, where the mitochondria are well preserved. In the upper layers they are enlarged and the mitochondria

disintegrate. Therefore, a close connection between the mitochondria and these keratohyaline granules is unlikely to exist (Cowdry³¹).

It has not yet been decided whether there is a connection between the mitochondria and the storage granules in general. Von Möllendorff,²⁵ Maximow and Bloom,²⁷ and many others considered them to be fundamentally different, whereas Oliver²⁹ and Bloom³² defended the theory of a transformation of mitochondria into storage granules, at least as far as the above-mentioned resorption granules in cells of renal tubules are concerned. Through the courtesy of Dr. Jean Oliver, I had the opportunity to study such cells and tubules with the PM. It is certainly true that the number of mitochondria is remarkably decreased in cells containing large resorption granules. But the evidence that the resorption granules are formed from fully developed mitochondria is not convincing. In the experiments with various cell suspensions it was observed that the storage granules can be very small in cells exhibiting relatively large mitochondria. The storage granules, therefore, must originate in particles which are much smaller than normal mitochondria.

An intermediate position between the two extreme opinions is taken by Claude,⁶ who assumed that the mitochondria and the secretory granules "constitute extreme forms in a continuous series of cytoplasmic elements." When dealing with the significance of the microsomes, this opinion shall be mentioned again.

A further observation to be discussed is the appearance of very small granules in the blisters of cells which are suspended in distilled water or in 0.05 M ammonia. Two similar observations are reported by Hogue²² and Chambers and Fell.³³ These particles presumably are identical to microsomes (Bayliss⁸) or to ultramicroscopic particles (Bensley⁴). Of course, Hertwig's original definition of microsomes as particles, the size of which is (under ordinary circumstances*) below the resolving power of a light microscope,¹⁵ leaves one undecided whether or not it includes several kinds of particles. As a matter of fact, two submicroscopic elements of different chemical composition have been recognized in liver cells (Lazarow³⁴). Since the microsomes measure from 60 to 150 μ (Claude⁶), it is obvious that they are scarcely visible under the ordinary microscope, as well as under the PM; but the slightest enlargement brings them within the range of visibility. This fact is utilized fully by the PM, which allows them to be observed without any shrinkage caused by fixation. Further-

* Words in parenthesis were added by the author.

more, the PM makes it possible to select blisters containing separated microsomes for observation.

A similarity between the microsomes and the secretory granules, as suggested by Claude,³⁵ could not be noticed in my experiments. The optical picture of microsomes, as well as their behavior in different mediums, is contrary to those of secretory granules. In recent studies, Claude⁶ observed the breakdown of mitochondria into small "microsome-like elements," and Baker³⁶ pointed out that microsomes are chemically very similar to the mitochondria.

Future investigations may help to decide whether there is a fundamental difference between mitochondria and microsomes, or whether microsomes are an intermediate stage in the formation of mitochondria *de novo*. The above-mentioned opinion of Claude⁶ with a slight modification, seems in best agreement with the sum of the numerous known facts. Accordingly, microsomes would have to be regarded as the original, undifferentiated particles, which, corresponding to the needs of the cell, may develop into mitochondria as well as into secretory granules.

The bright intraprotoplasmatic vacuoles which occur exclusively in cells of old suspensions and of disintegrating tumors have nothing in common with the blisters.¹ They are probably the result of protoplasmic autodigestion, as suggested by Cowdry.¹⁶

There seems to be little doubt that the large inclusions in tumors represent necrotic tumor cells in which the remains of the disintegrated nuclei are sometimes still visible as dark fragments. The different stages of this necrophagocytosis can be observed in a suspension of a highly necrotic V2 carcinoma. The dead cell body is enveloped by the protoplasm of a living tumor cell. The large granules, stained with neutral red, are probably storage granules which developed before the cell had died.³⁷

SUMMARY

A study of the protoplasmic constituents of various cells with the phase microscope has led to the following observations and conclusions:

The mitochondria appear as small gray or black, indistinctly outlined, spherical or rod-like granules. Normally, they appear as solid particles, but in distilled water and in dilute solutions of ammonia they enlarge greatly, and fluid accumulates between a superficial interface membrane and the slightly swollen "body" of the mitochondrion. In normal cells some of the mitochondria exhibit brownian movement.

Mitochondria react readily when the cell is exposed to adverse environmental conditions. They are not fragile, however, and often may be seen for some time after the mechanical destruction of the cell.

Storage granules are usually spherical or slightly irregular and have a brilliant blue hue. Their fluid contents are surrounded by a thin membrane, which may be seen as a "shell" after the destruction of the granules. They may enlarge in a number of ways: by the accumulation of waste products (degeneration granules), by preparing cellular products for secretion (secretory granules), or by resorption (resorption granules). The zymogen granules, the hyaline droplets in renal tubular cells, and the irregular keratohyaline granules of squamous cells belong to this group of cytoplasmic constituents.

The microsomes cannot be seen in the cellular cytoplasm except in the blisters produced by distilled water or ammonia. These visible microsomes are probably greatly swollen. It is conceivable that microsomes may be undifferentiated precursors of mitochondria and storage granules.

The protoplasm of cells which have been in suspension for a long time contains irregular degeneration vacuoles. They are bright and have nothing in common with blisters.

No Golgi apparatus could be identified with the phase microscope in these experiments.

Several types of experimental tumor cells often contained large protoplasmic inclusions which seemed to represent phagocytized disintegrating cells.

It appears that the protoplasm is a colloid in a polyphasic state of sol-gel balance, and that this state may change very easily.

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DESCRIPTION OF PLATES

PLATE 104

- FIG. 1. Isolated kidney cell of the frog. The large, bright elements with black contour are storage granules; the indistinct gray granules are mitochondria. On the left there is a single fat droplet. The cellular membrane is not in focus. PM. $\times 1400$.
- FIG. 2. Brown-Pearce carcinoma cells in distilled water. The nuclei are hazy and moderately enlarged, and the mitochondria are swollen. The nucleoli of the two cells in the middle are out of focus. PM. $\times 1400$.
- FIG. 3. A group of unchanged Brown-Pearce carcinoma cells for comparison with Figures 4 and 5. PM. $\times 1400$.
- FIG. 4. The same group of carcinoma cells shown in Figure 3, 3 minutes after the replacement of the physiologic saline solution by distilled water. The nuclei are swollen and hazy, the nucleoli are indistinct (out of focus), and blister formation is accelerated. Enlarged, black mitochondria may be seen. PM. $\times 1400$.
- FIG. 5. The same group as shown in Figures 3 and 4, 4 minutes after further replacement of the distilled water by physiologic saline solution. The nuclei are smaller than in Figure 4; the chromatin network shows the same arrangement as in Figure 3, and the nucleoli have reappeared. The mitochondria have regained their original size. PM. $\times 1400$.
- FIG. 6. C₃H sarcoma of the mouse. The mitochondria and the storage granules are easily distinguishable from each other. The cell on the left contains two rod-like mitochondria near the lower pole of the nucleus. PM. $\times 1400$.
- FIG. 7. The effect of distilled water on mitochondria, which float freely in the suspension medium after mechanical destruction of frog kidney cells. The mitochondria are markedly enlarged and vesicular; the black, distinct thickening of the wall (see text) is readily visible in many of the vesicles. PM. $\times 1400$.
- FIG. 8. C₃H sarcoma cell after replacement of the distilled water by physiologic saline solution. The nucleus has lost its hazy structure and the chromatin network has reappeared. The mitochondria are still round, but not markedly enlarged. PM. $\times 1400$.

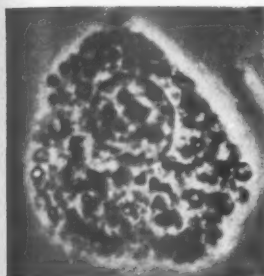


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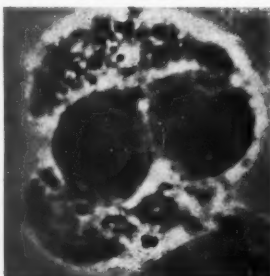
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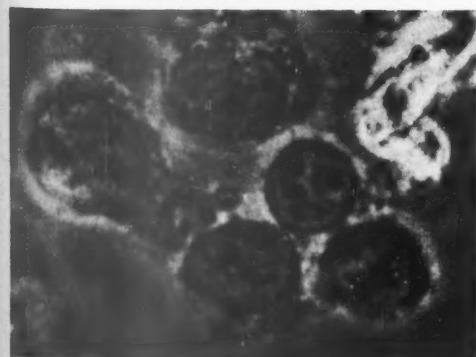
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Zollinger

Phase Microscopy, Mitochondria

PLATE 105

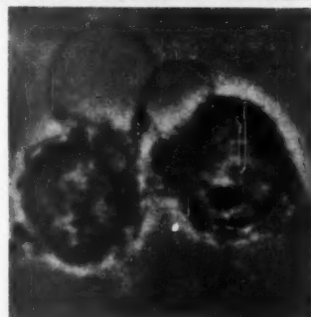
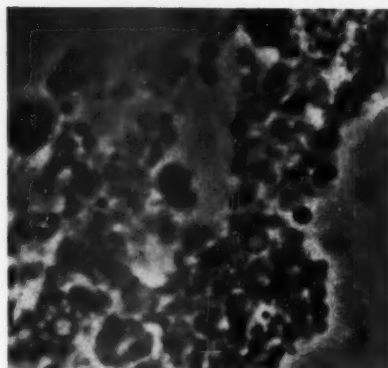
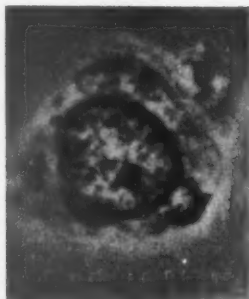
- FIG. 9. Kidney cells of the frog in physiologic saline solution, to which distilled water had been added just before the picture was taken. The mitochondria in the protoplasm are still rod-like and small, whereas they are greatly enlarged in the blister contents. Besides the enlarged mitochondria, the blister contains two very small particles (microsomes). PM. $\times 1400$.
- FIG. 10. Brown-Pearce carcinoma cell in saline solution of pH 4.0. The nuclear membrane has become brilliant; the chromatin network is very dense; the nucleolus is enlarged; and the protoplasm has shrunk. PM. $\times 1400$.
- FIG. 11. Large, vesicle-like mitochondria and storage granules of frog kidney cells in 0.5 M ammonia. The cellular membrane has been destroyed completely, and the granules are markedly swollen. PM. $\times 1400$.
- FIG. 12. Ciliated epithelial cells of the frog, treated with 0.1 M ammonia, and washed afterwards in physiologic saline solution. Most of the mitochondria again are small, but where they had merged before, they did not decrease in size (large black dots). PM. $\times 1400$.
- FIG. 13. Brown-Pearce carcinoma cells, after having been at 6° C. for 3 hours. The mitochondria and the storage granules are very small; the cells show marked potocytosis. PM. $\times 1400$.
- FIG. 14. V2 carcinoma cells killed by pressure on the coverslip. The mitochondria have disappeared; the storage granules are well preserved, but very small. The nuclear membrane is no longer visible. PM. $\times 1400$.
- FIG. 15. Numerous irregular, black granules in an epithelial cell of the human mouth. The nucleus is slightly shrunken. Of note is the thick, irregular cellular membrane. PM. $\times 1400$.
- FIG. 16. A cell of the same type as shown in Figure 15. The granules are larger, shiny, and partly spherical. PM. $\times 1400$.
- FIG. 17. A cell from a Shope papilloma of the rabbit, containing granules of the same type as shown in Figure 16. The nucleus is out of focus. PM. $\times 1400$.



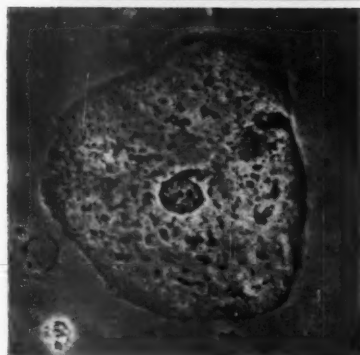
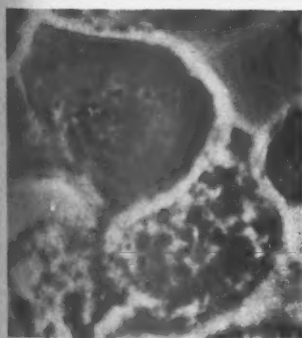
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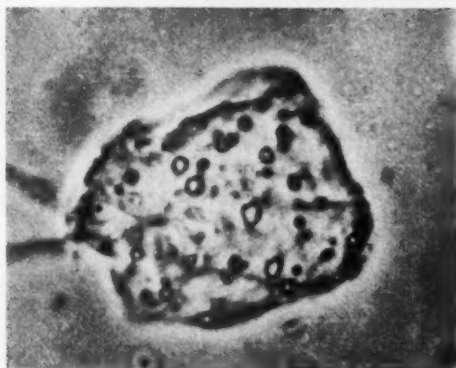
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Zollinger

Phase Microscopy, Mitochondria

PLATE 106

FIG. 18. Blister in a kidney cell of the frog, in distilled water containing two tiny, black granules (microsomes). PM. $\times 1400$.

FIG. 19. Liver cell of the frog filled with fat droplets and a few storage granules. PM. $\times 1400$.

FIG. 20. Brown-Pearce carcinoma cell with a large inclusion body. The bright ring around the body indicates that the latter is quite spherical. PM. $\times 1400$.

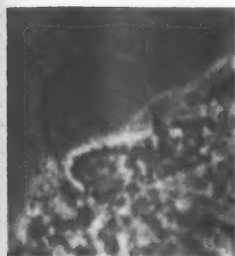
FIG. 21. Brown-Pearce carcinoma cells after having been in suspension at 37° C. for 3 hours. Blister formation can be seen in every cell. In addition, the cell on the left contains several vacuoles. The brilliant storage granules and the black or gray mitochondria are easily distinguishable from each other. The nucleoli are large, and the chromatin network is plump. PM. $\times 1400$.

FIG. 22. Brown-Pearce carcinoma cells of a fresh suspension. At the upper left there is a necrotic cell with a bright diffraction ring; next to it there is a degenerated cell with enlarged storage granules. The cell below shows vacuoles. PM. $\times 1400$.

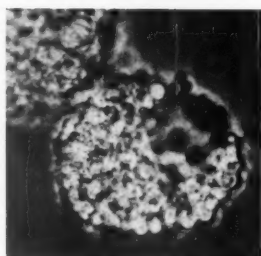
FIG. 23. Rabbit sarcoma cell with two paranuclear "vacuoles" and a large blister. PM. $\times 1400$.



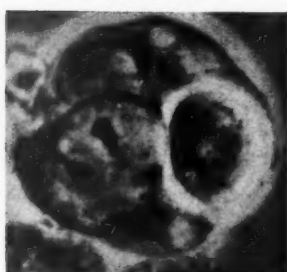
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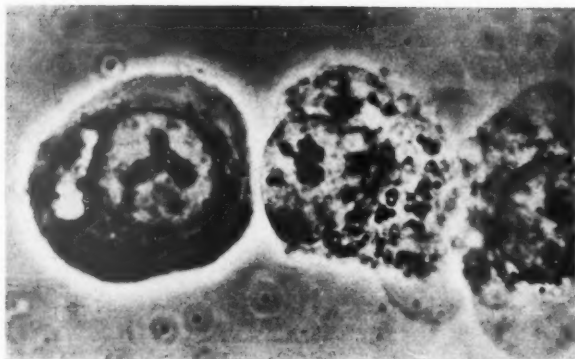
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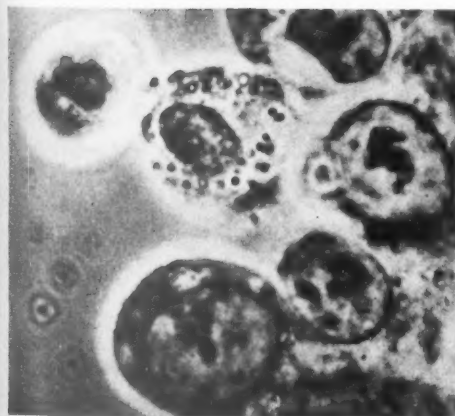
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Zollinger

Phase Microscopy, Mitochondria

MELANOMAS OF CHILDHOOD *

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It has become apparent over a period of years that even when a histologic diagnosis of malignant melanoma has been made in children the clinical behavior rarely has been that of a malignant tumor. The disparity in behavior of the melanomas of adults and children, despite the histologic similarity of the lesions occurring in the different age groups, is obviously a matter of fundamental importance and the following questions immediately arise: Does the histologically malignant melanoma of children differ in any structural detail from that of adults? Can the clinical behavior of these lesions be predicted from their histologic structure? What, if any, are the factors known to influence the clinical behavior? Should the melanomas of children be treated any differently from the melanomas of adults?

MATERIAL

In a search of the files of the Memorial Hospital for instances of malignant melanoma in children, it soon became apparent that the diagnosis had been made with far greater frequency 20 or more years ago than in the past decade. This difference was quickly accounted for in the usual structure of the benign pigmented nevi of children as contrasted with that of the benign nevi of adults. In more recent years, the criteria for the diagnosis of malignant melanoma had become clarified to the extent that histologic features of the nevus of childhood, formerly regarded as stigmata of malignant change, were no longer so considered. However, there remained a group of cases in which a diagnosis of malignant melanoma seemed histologically sound. Over a period of years, the qualification has been added to reports of such lesions that they probably would not behave as malignant tumors. In order to distinguish these lesions both from the malignant melanoma of adults and the unequivocally benign nevus of childhood, the term "juvenile melanoma" has been adopted. The term "melanoma" in this paper, as in common usage, has been applied only as an abbreviation for malignant melanoma.

The material for this study is comprised of 13 cases † diagnosed histologically as juvenile melanoma during the past 13 years and occurring in children ranging in age from 18 months to 12 years. For

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† Submitted from the Mixed Tumor Service of the Memorial Hospital.

purposes of comparison, a group of melanomas occurring in young adults of from 14 to 19 years of age also was reviewed. In addition, 50 consecutive cases of benign nevus occurring in children ranging in age from 1 month to 12 years were included in the comparative study. Blue nevi (Jadassohn) and Mongolian spots were not included in this study since they form a recognizable entity usually easily segregated from malignant melanomas both in histologic appearance and in their generally benign clinical behavior.

Hematoxylin and eosin preparations of all lesions were available for study; in some instances silver stains and Masson's trichrome preparation also were used.

CLINICAL FEATURES

In the group of childhood melanomas (juvenile melanomas) there were 5 males and 8 females. Three were less than 2 years of age; one was 3 years of age; one, 5 years old; and the remaining 8 patients ranged in age from 8 to 12 years. The clinical appearance was varied: 10 of the 13 patients had lesions under 1 cm. in diameter and only 3 lesions were between 1 and 3 cm. In a few, the lesions were described as being smooth with sharply delimited edges, but in the majority they were verrucous with irregular margins (Figs. 1 to 4). All were elevated above the skin surface. The color was described as pink to red in 5 whereas 7 varied from brown to black. One lesion was said to have been subcutaneous. None was described as hairy.

The lesions had been noted for the duration of life in 6 cases but were said to have existed for from 6 weeks to 4 years in 7 cases. Three of the patients were presented for treatment within 1 year of the first appearance of the lesion. There was a history of gradual increase in size in all cases except one in which there was rapid growth for 6 weeks only.

Five of the lesions occurred on the face, one on the trunk, 2 on the upper extremity, and 5 on the lower extremity; only one of the latter category occurred on the sole. The parents of all these children stated that the lesions were in locations where they were frequently traumatized during the course of daily activities but none gave a history of frequent bleeding and none of the lesions was grossly ulcerated at the time of examination. Treatment consisted only of local surgical excision in all cases; in one case a group of obviously metastatic nodes was later removed from the groin.

All but one of the 13 children are alive and have shown no evidence of recurrence either locally or in drainage sites. They have been fol-

lowed clinically for periods up to 13 years. Only 2, both female, have been followed for as short a time as 3 years and both of these have now passed their menarche. The remaining 10 have been seen regularly for from 5 to 13 years; 6 of these have passed the age of puberty.

One of the 13 cases had been clinically malignant and the child is dead. This one fatality occurred in a female child whose lesion was first noted at the age of 12 years; there had been no development of secondary sex characteristics and she had not menstruated. This lesion occurred on the sole of the foot but was not described as involving the skin. After rapid growth over a period of 6 weeks, a soft white tumor, 2 cm. in diameter, was resected from the plantar fascia. One month after the initial excision there was a bulky local recurrence, thrombosis of the femoral vein, and metastasis to inguinal lymph nodes. Within 4 months the child was dead of generalized metastases.

HISTOLOGIC FEATURES

The epidermis was present in the sections studied in 12 of the 13 cases and was in all instances altered in a characteristic manner. Frequently there was hyperkeratosis and occasionally patchy parakeratosis. The epidermis immediately over the bulk of the tumor often was acanthotic and showed spongiosis, sometimes to so marked a degree that small intra-epidermal vesicles were present. There was superficial ulceration of the epidermis of 2 of the lesions; neither of these was malignant clinically. The rete pegs in 7 of the lesions were irregularly elongated and extended rather deep into the dermal tumor.

The most distinctive feature of the epidermal change was found in the basal layer, which was not uniformly palisaded as in the normal skin. The continuity of the basal layer was interrupted by scattered cells or groups of cells which were irregularly enlarged and distended by uniform fine brown granules. Similar isolated cells also were occasionally scattered irregularly in the acanthotic malpighian layer. These enlarged pigmented cells often were increased twice or more in size over normal basal cells; the nuclei were of varied size but were mainly large and vesicular. There was a loss of cohesion between these altered cells and the adjacent cells of the epidermis. This change, often referred to as the junctional or dermo-epidermal change, occurred diffusely over the entire surface of the tumor, but often there was added to the diffuse change a more distinctive alteration in which islands of large pigmented cells formed bulbous knobs and pegs which extended down into the dermis (Figs. 5 and 6). In places, the extensions from the epidermis seemed to be bounded by an intact basement membrane,

but in all lesions it was possible to trace direct continuity between them and the cells forming the dermal portion of the tumor.

Nine of the 13 cases presented a histologic appearance which in most respects was indistinguishable from the adult type of malignant melanoma. In 3 of these the lesions were relatively superficial and had infiltrated only to the level of the mid-dermis; in 6 there was infiltration through the entire dermis. The structure varied not only in the different lesions but also in any one lesion. The large cells distended with fine pigment, described in the epidermis, formed long projections into the dermis. These cells at times assumed a definite spindle shape in the infiltrating portion of the tumor. In several there were compact clusters of spindle cells, but this structure was predominant in only one case of this series, that is, the one fatal case. In the remainder, spindle cells were interspersed among large acidophilic cells which more often formed the bulk of the tumor. These cells were varied in size but were always large, rounded or polygonal, with vesicular nuclei and large acidophilic nucleoli (Fig. 5). There was either alveolar or perivascular arrangement of the cells.

In one feature alone some of these lesions were distinctly different from the malignant melanoma of adults. In 8 of the 9 cases just described, giant cells were present both in the epidermal and dermal portion of the tumor (Figs. 7 and 8). In 5 cases there were small to moderate numbers of these cells, but in 3 cases giant cells were present in such large numbers as to constitute the most outstanding feature of the lesion. These giant cells were totally unlike those formed by fused nuclei seen so commonly in the benign nevus. They were most prominent in the basal layer of the epidermis or in the superficial part of the dermal tumor and were either multinuclear or mononuclear. In the multinucleate cells the number of nuclei varied from four to six; generally they were in peripheral arrangement but occasionally were clumped in the center of the cells. The cytoplasm was acidophilic and sometimes granular. Pigment was seldom seen in the giant cells but commonly they contained vacuoles suggesting fat. Most of the giant cells were round or oval but often there were stellate cytoplasmic processes, particularly in those connected with the epidermis (Fig. 8). Silver stains have failed to show argyrophilic processes on these or other cells of the tumor; nor do trichrome stains indicate that they have origin in muscle.

In 3 cases, the dermal portion of the tumor was composed entirely of spindle cells, different principally from those described above in the large cytoplasmic content of the cells and the rather orderly inter-

lacing bundles of cells (Fig. 9). This structure bore strong resemblance to epidermoid carcinoma, particularly of the spindle cell type. The junctional change so constant a feature of the entire series also was present in this part of the group.

One case was especially different from the other 12. A 10-year-old boy had a black lesion on the lip, present since birth. He has been followed for 7 years and there has been no recurrence. This lesion had essentially the structure of a simple benign intradermal nevus with clusters and strands of cells extending into the subcutaneous fat. The distinctive feature was the almost uniform enlargement of each cell to a diameter three or four times that of an ordinary nevus cell. The increase in size was primarily in the amount of cytoplasm, which was peppered with fine melanin granules. The nuclei also were enlarged and hyperchromatic, but irregularly so. There were acidophilic nuclear inclusions which far outnumbered those in other lesions of this series. At the periphery of only three other lesions of this series were there cords and nests of small round tumor cells.

Mitotic figures were not prominent in any of these lesions but occasionally could be identified without any great difficulty both in the epidermal and dermal portions of the tumors.

Pigment was present in all of the lesions but only 3 were heavily pigmented. Melanin was far more prominent in the enlarged cells at the dermo-epidermal junction than in the other portions of the tumor but was present also in scattered tumor cells of the dermis, in the malpighian layer of the epidermis, in the parakeratotic scales and vesicles, and in the dermal chromatophores (Fig. 6). The differences in color noted clinically could not be correlated with differences in pigment content. Actually 2 of the most pigmented lesions were clinically red. The color variations were most easily accounted for on the basis of the vascularity of the tumor; that is, those that were red showed greater vascularity rather than less pigment.

The cutaneous appendages often remained intact in the tumor. The basal layer of the hair follicles participated in the junctional change which occurred in the epidermis, but to a lesser degree. The sebaceous and sweat glands were not altered except by distortion due probably to pressure of the surrounding tumor.

Associated with juvenile melanomas were inflammatory changes consisting in a few cases simply of a sparse infiltrate of lymphocytes and plasma cells at the periphery of the lesion. In other lesions the infiltrate was more intense and involved the tumor itself as well as the tissues surrounding the tumor. In 2 cases in which vesiculation and

ulceration of the epidermis were noted there were also polymorphonuclear neutrophils and eosinophils in the infiltrate.

In most of the tumors there was diffuse edema involving not only the epidermis but also the dermis, particularly the papillary layer. In places there seemed to be an almost complete dissolution of the basal layer and the tumor cells appeared to be floating in the edema fluid of the dermis. The capillaries of the papillary layer were dilated and engorged. The lymphatics of the dermis were also dilated, particularly at the dermo-epidermal junction.

Differentiation of Juvenile Melanoma from Benign Nevus of Childhood

The histologic sections of 50 unselected benign nevi of children were studied for purposes of comparison with juvenile melanoma. These nevi were removed chiefly for cosmetic reasons from children ranging from 1 month to 12 years of age, occurred in the skin in almost all regions of the body, and ranged from very small macules to large lesions that covered almost the entire trunk. All of these children are alive and none has shown recurrence over periods up to 7 years.

The ratio of incidence of juvenile melanoma and of the ordinary benign nevi of childhood is difficult to determine inasmuch as usually only the nevi of unusual clinical appearance are removed. However, an approximation of the relative incidence may be gathered from the fact that over a period of about 6 years 100 pigmented nevi of children were removed surgically; of these there were 8 juvenile melanomas, or a ratio of 1:12.

In contrast to the pleomorphic structure encountered in the group of juvenile melanomas, the benign nevi of childhood were monotonously alike, in most instances, in their histologic structure. The epidermis covering the nevus was generally thin but showed segments of acanthosis. There was increased pigmentation in all layers but the pigment was most concentrated in the basal layer. In 49 of the 50 lesions (98 per cent) there were scattered, somewhat enlarged, pigmented cells in the basal layer singly as well as in nests (Fig. 10) which extended into the dermis. The projecting nests were sometimes still bounded by a compressed rim of basal cells. One of the 50 lesions showed no alteration of the epidermis overlying the nevus.

As a rule, the benign nevus of children was far more cellular than the ordinary nevus of adults. The upper segments of the nevus were crowded with closely packed pigmented nevus cells which were of uniform size and shape; in the lower segments of the tumor there was

gradual diminution in the amount of pigment and in the size and number of cells, as well as increase in fibrous tissue. The deeper segments of the benign nevus in children were often composed of delicate strands of small nonpigmented cells surrounded by large collagenous bands. It also was noted that the structures resembling Meissner's corpuscles (lames foliacées), so commonly found in adult nevi, were practically absent in this series.

There are, then, definite cellular features of distinction between the juvenile melanoma and the ordinary benign nevus of children: (1) The pleomorphic structure of the juvenile melanoma is in contrast to the monotonous structure of the benign nevus of children; (2) In juvenile melanoma there are bizarre mononuclear or multinuclear giant cells totally unlike those formed by fused nuclei in the benign nevus; (3) The junctional change so prominent in the benign nevus is comprised of cells which are uniform, small, and closely packed whereas in the juvenile melanoma these cells are pleomorphic, larger, and form looser projections in the dermis; (4) Mitotic figures, occasionally seen in the juvenile melanoma, are rare in the ordinary nevus.

While the details of the problems of the morphogenesis of nevi are beyond the scope of this paper, certain features of differentiation between benign nevi of children and the corresponding lesion of adults are worth noting. There appears to be a remarkable difference in the incidence of junctional change in the nevi of the two age groups. This alteration was present in 98 per cent of the children included in this study and is in contrast to the reports of Allen¹ of 12 per cent and of Montgomery and Kernohan² of 25 per cent in their studies of adults. The pronounced cellularity in the nevi of childhood has led to the erroneous diagnosis of malignant melanoma just as the cellularity of hemangiomas of infancy has led to the diagnosis of angiosarcoma by those not familiar with the natural evolution of these lesions.

Differentiation of Juvenile Melanomas from Adult Melanomas

In view of the radical contrast in behavior between juvenile and adult melanomas, it seemed of interest to determine the life history and possible histologic variations of melanomas occurring in an intermediate age group. Accordingly, a series of 17 melanomas occurring in patients ranging in age from 14 to 19 years was used for comparative study. In this group there were 5 males and 12 females. Three of the lesions occurred on the face or neck; 5 on the trunk; 2 on the upper extremity, and 7 on the lower extremity (none on the sole). There was a history in all that the lesions had been growing for from 1 to 2 years

before local excision; some of these lesions had been present for a lifetime. All of the female patients had undergone menarche from 3 months to 4 years before the removal of the tumor. In several of the females there was a definite history that the pigmented cutaneous lesion had increased two to three times in size since the onset of menstruation which had occurred only 3 to 4 months prior to the removal of the tumor.

In the group of 13 "juvenile melanomas," only one patient is dead (7.7 per cent) whereas in a similar group of melanomas of 17 young adults, 12 are dead (71 per cent), the fatalities having occurred within 6 to 18 months after the initial diagnosis. An analysis of the 5 living patients reveals one with metastasis that has survived for 4 years. Four (23.5 per cent) have survived for periods of 5, 9, 11, and 17 years, respectively. The average 5-year survival for adults of all ages, as determined recently in a series of 595 cases,³ is 9.7 per cent. There is at least a suggestion in these figures, obviously in need of confirmation by a larger series, that perhaps melanomas occurring even in an intermediate age group carry a more favorable prognosis than those occurring at a later age.

In the fatal cases, ranging from 14 to 19 years, the variations in structure were so great that it was not considered possible to correlate prognosis with histologic appearance. However, several features of this group bear noting. In only one of the adults (Fig. 11) were there giant cells of the type that were identified in approximately one-half the group of juvenile melanomas. This patient has now survived for 5 years. Similar cells have been noted occasionally in adult nevi (not included in this study).

Although there was some tendency toward less pigmentation in the juvenile melanomas, this feature was too inconstant to be of diagnostic or prognostic significance. Mitotic figures were more numerous in the melanomas of the intermediate age group but they were present sufficiently often in the juvenile melanomas to make this latter lesion a definite exception to the rule that mitotic figures in nevi are evidence of malignant melanoma.

A generally appreciated feature that was again demonstrated was the lack of correlation between the depth of the local cutaneous infiltration of the lesions and the ultimate outcome. The lesions in several of the fatal cases of young adults were extremely superficial and some had a qualitative histologic appearance far less malignant than many of the nonfatal juvenile melanomas.

In general, it was concluded that differentiation histologically between the juvenile and adult melanomas could not be made with certainty in most cases. The one feature, found in almost one-half the cases of juvenile melanoma, that seemed to permit a histologic distinction from adult melanoma, was the presence of giant cells (Figs. 7 and 8). In view of the survival of patients having this type of tumor, these have been regarded as an indication that the lesion is benign. This is so despite the fact that, except for the giant cells, such lesions have all the histologic criteria for the diagnosis of malignant melanoma.

INCIDENCE OF MELANOMAS IN CHILDHOOD

Contrary to the general impression of the frequency of occurrence of malignant melanoma in children, a review of the recent medical literature reveals very few reports substantiated either histologically or by fatal outcome. Wells⁴ stated that "Although pigmented moles are frequently present at birth, they rarely become malignant before birth or even in infancy." He accepted only the case of Coe⁵ as a true congenital melanoma; this lesion occurred on the scalp of a newborn infant, grew rapidly, metastasized to nodes, and caused death in 4 months. Milian, Périn, and Brunel⁶ reported an instance of melanoma occurring in the parietotemporal region of the male, 12 years of age, but neither photomicrographic evidence nor follow-up data are presented as corroboration of the diagnosis. The case of Périn and Blaire,⁷ occurring on the cheek of a child, 3 years of age, appears histologically to have been melanoma but the child died of bronchopneumonia following whooping cough 7 months after the initial excision of the lesion so that clinical evidence of its malignant course is lacking.

Sweet and Connerty⁸ have reported a bulky tumor replacing the genitalia in an infant that also had a bathing trunk nevus; this child died shortly after birth and had hepatic and pontine metastases. The pontine lesion was heavily pigmented and the authors felt that the logical diagnosis was probably malignant melanoma. The recent report of Russo⁹ in which osseous metastases are described in 2 children, 5 weeks and 3 years of age, is not substantiated by photographic proof of the diagnosis, and the possibility comes to mind that these 2 cases might represent neuroblastomas rather than melanomas. The lesion in his third case, occurring in a Negro female, 5 years old, might well be a melanoma but this child has been well for 3 years after the excision of the tumor.

Webster, Stevenson, and Stout,¹⁰ however, mentioned 10 cases of

histologic melanoma which occurred in children under the age of 10 years. Only 2 cases are detailed specifically in their paper but neither is recorded as having been fatal. The outcome of the other cases is not stated but the authors do mention that lesions in children giving the histologic appearance of malignant melanoma rarely metastasize.

Callender, Wilder, and Ash,¹¹ in a review of 1600 ocular melanomas, recorded only 2 instances in patients from 0 to 9 years and 13 from 10 to 19 years. Although their follow-up data are admittedly incomplete, the youngest patient to die in their series was 19 years of age.

In the current study, the histologic diagnosis of juvenile melanoma has been made in 13 cases while only one of these has been clinically malignant. This one fatal case, occurring in a 12-year-old girl, was distinctly different histologically as well as clinically from the group as a whole. The tumor was composed entirely of nonpigmented spindle cells and involved primarily the plantar fascia (Fig. 12). Unfortunately, a section of overlying skin was not submitted with the primary tumor, but the metastatic lesions in inguinal lymph nodes were of the pleomorphic structure generally encountered in melanomas.

A case which was both clinically and histologically malignant recently was submitted to this laboratory by Dr. Bjarne Pearson of the Department of Pathology of the University of Vermont. This lesion occurred in a 9-year-old female child who was normally developed and showed no precocious sexual features. The pigmented lesion on the knee was only 4 mm. in diameter at the time of removal and, while it had been present for several years, growth had occurred over a period of only a few weeks. The cells of the primary lesion in this instance contained large, irregular, hyperchromatic nuclei with prominent vacuoles and acidophilic inclusions which would justify the diagnosis of malignant melanoma, regardless of the age (Fig. 13). Bilaterally, the inguinal nodes showed a few clusters of metastatic cells in the peripheral sinuses. Although the follow-up in this child has been only for a period of 6 months and it is not possible to predict the outcome, it has been noted that generalized dissemination of the tumor has become evident in the fatal cases of young adults and in the one fatal case among the children within a very short time after the diagnosis has been made. It seems possible, however, that in some cases metastases to regional nodes in children need not always indicate a fatal termination. This peculiarity of melanomas in children would seem to be indicated by the case included in the report of Webster, Stevenson, and Stout¹⁰; this child, 8 years old, after local excision of

a black lesion on the shoulder and subsequent metastases both to skin and cervical lymph nodes which were resected, had survived at least 12 years without further recurrence.

FACTORS INFLUENCING CLINICAL BEHAVIOR OF JUVENILE MELANOMAS

Inasmuch as there is a lack of constant morphologic evidence with which to explain the usually benign clinical behavior of histologically malignant melanoma occurring in childhood, an explanation based on sex-linked hormonal control would seem logically feasible. The peak of incidence of malignant melanoma occurs between the age of 40 and 60 years. Despite the fact that both cutaneous and ocular melanomas are relatively uncommon in younger age groups, there is too sharp a rise in mortality once the age of puberty is passed to be attributable to a general increase in incidence of cancer with age.

There is, moreover, in our experience, frequent recurrence of the clinical information that the growth of pre-existing nevi is greatly accelerated at the time of, or shortly after, puberty. At times these cases will follow a rapidly fatal course out of all proportion to the morphologic appearance of the lesion (Fig. 14). Two cases of malignant melanoma^{5,8} have been reported in the newborn in which ante-natal metastases have occurred; a variety of hormonal influences exist during this period which do not ordinarily obtain thereafter. There is some evidence¹⁰ that even though metastasis may occur in childhood, an inhibitory factor may exist before puberty to hinder either further dissemination or reception of metastatic cells by the viscera. Presumptive though this evidence may be, an intensive investigation of the possible influence of sex-linked hormonal alterations on the activation of melanoma seems mandatory.

SUMMARY AND CONCLUSIONS

Of 13 cases of juvenile melanoma in this series, only one (7.7 per cent) has had a clinically malignant and fatal course despite the similarity of the juvenile lesions to the malignant melanoma of adults.

The juvenile melanoma may be distinguished histologically from adult melanoma in about one-half the cases by the presence of giant cells in the former which seldom occur in the latter.

There is a precipitous rise in the capacity of melanomas to metastasize after puberty despite the histologic similarity to the usually non-metastasizing juvenile melanoma.

The possible influence of sex-linked hormonal activation of the growth capacity of melanomas at the age of puberty seems a logical conclusion.

Accordingly, since metastases from juvenile melanomas occur only rarely, conservative surgery, rather than the radical surgery usually indicated for adult melanomas, seems justified.

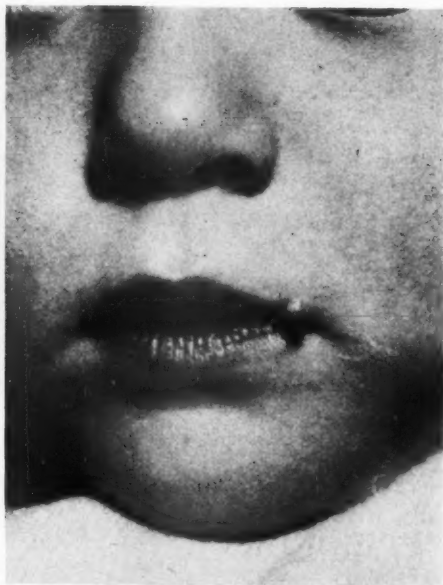
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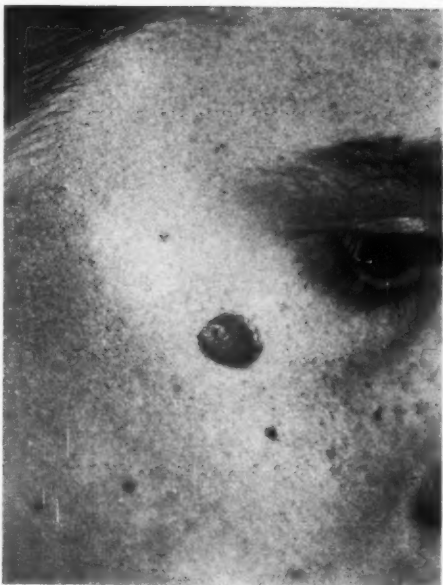
DESCRIPTION OF PLATES

PLATE 107

- FIG. 1. Male, 20 months of age. Smooth red lesion on cheek present since birth.
- FIG. 2. Female, 20 months old. Smooth black lesion on lip noted for 2 months.
- FIG. 3. Male, 5 years old. Verrucous brown lesion on chest present for 6 weeks.
- FIG. 4. Female, 4 years of age. Rough black lesion on cheek noted for 1 year.



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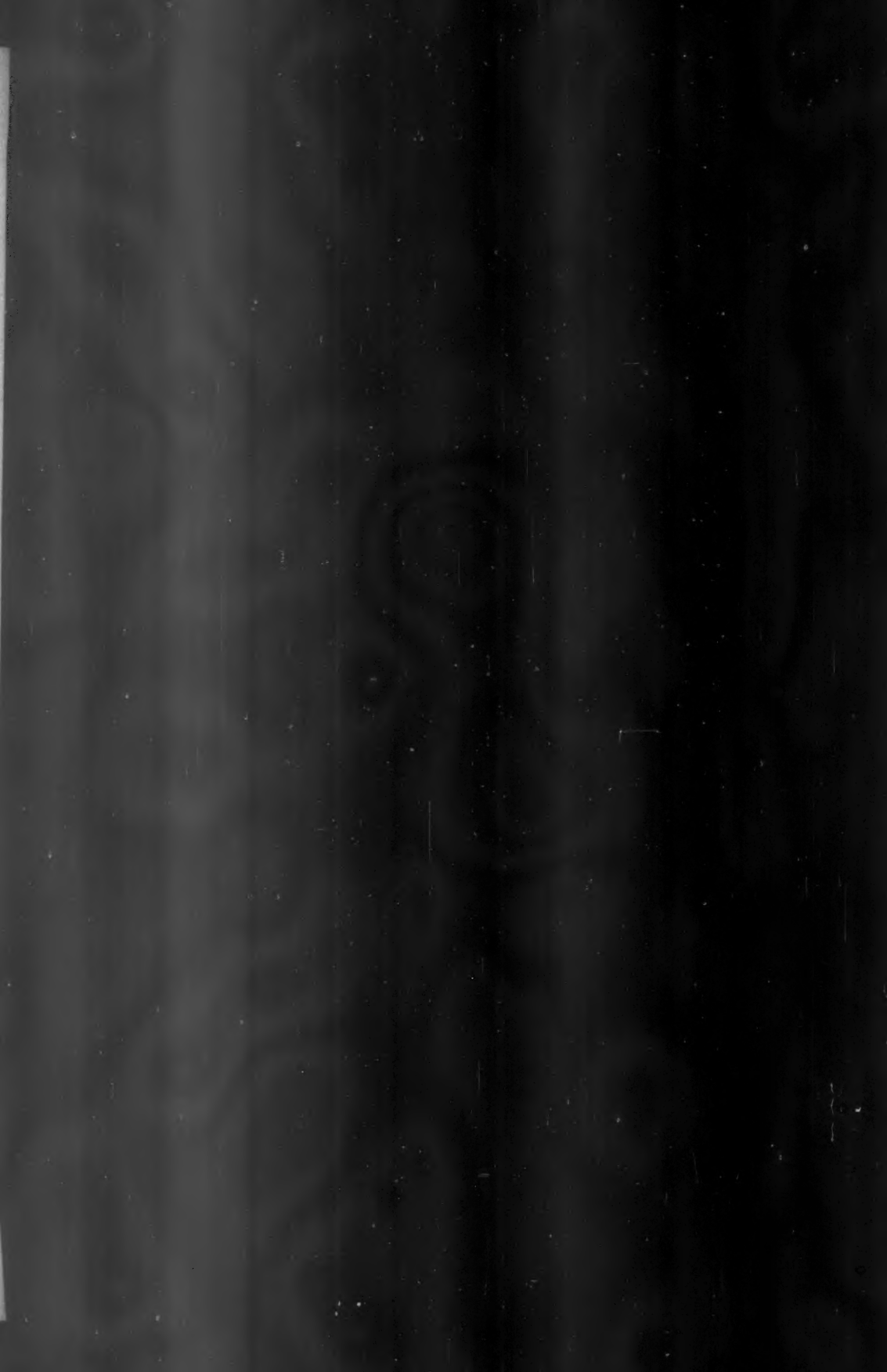
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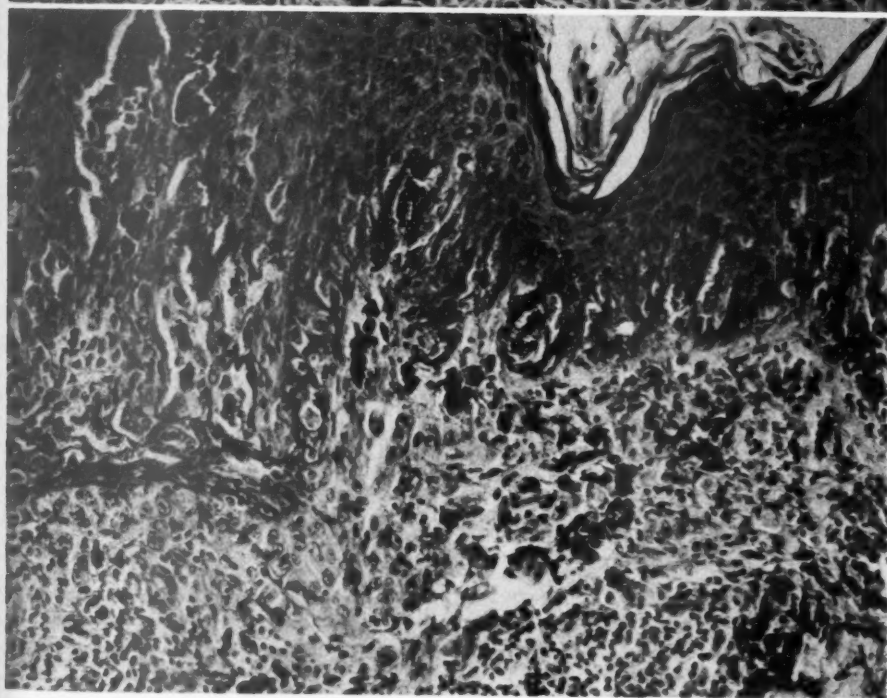
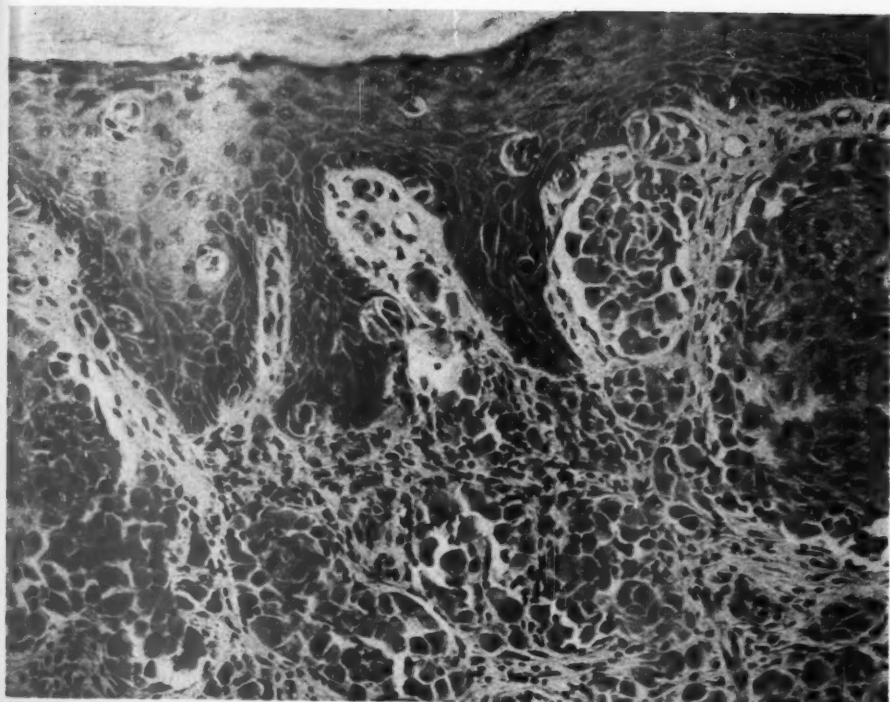
Melanomas of Childhood

PLATE 108

FIG. 5. Junctional alteration overlying juvenile melanoma formed by large pleomorphic acidophilic cells. Hematoxylin and eosin stain. $\times 220$.

FIG. 6. Heavily pigmented tumor on the thigh of an 11-year-old female, showing junctional alteration and pleomorphic infiltrating tumor. Hematoxylin and eosin stain. $\times 180$.





Spitz

Melanomas of Childhood

PLATE 109

FIG. 7. Giant cells at dermo-epidermal junction and upper dermis. Hematoxylin and eosin stain. $\times 550$.

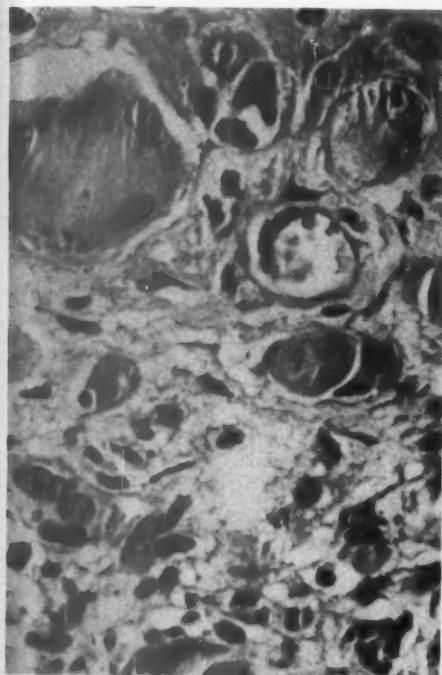
FIG. 8. Giant cells in the infiltrating portion of a juvenile melanoma. Hematoxylin and eosin stain. $\times 550$.

FIG. 9. Predominantly spindle cell tumor in a male, 20 months old. (From the same case as Fig. 1.) Hematoxylin and eosin stain. $\times 180$.

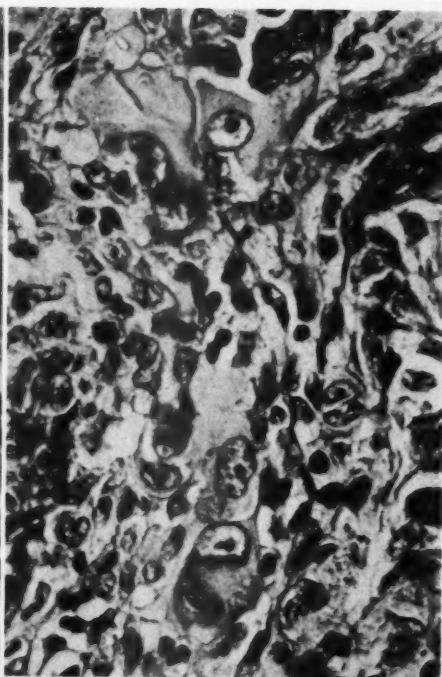
FIG. 10. Benign nevus in a child, 9 months of age. Hematoxylin and eosin stain. $\times 180$.



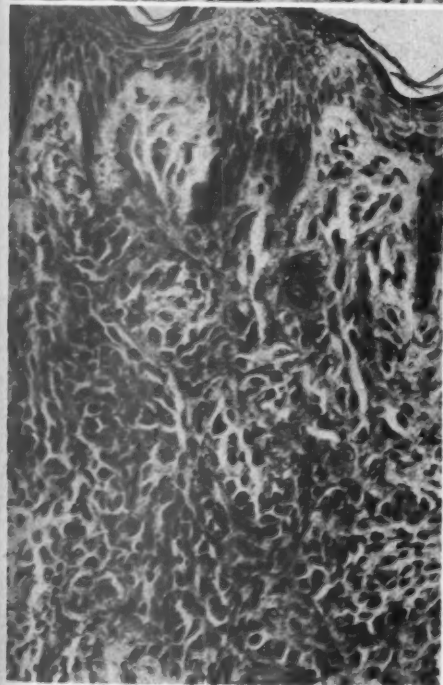
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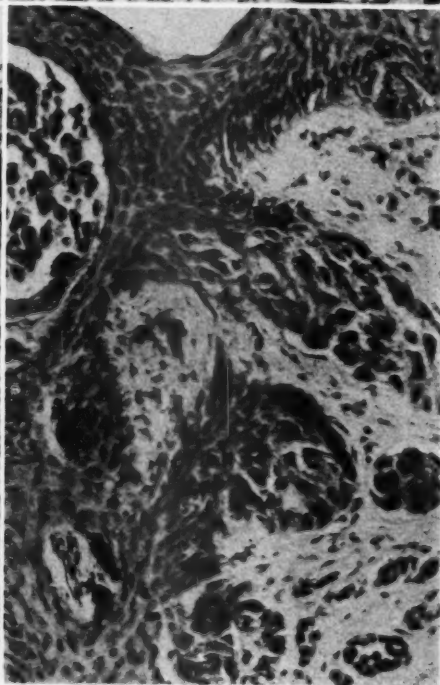
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Spitz

Melanomas of Childhood

PLATE 110

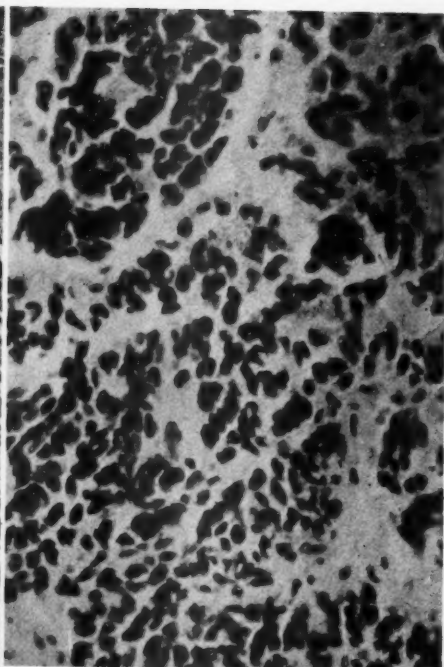
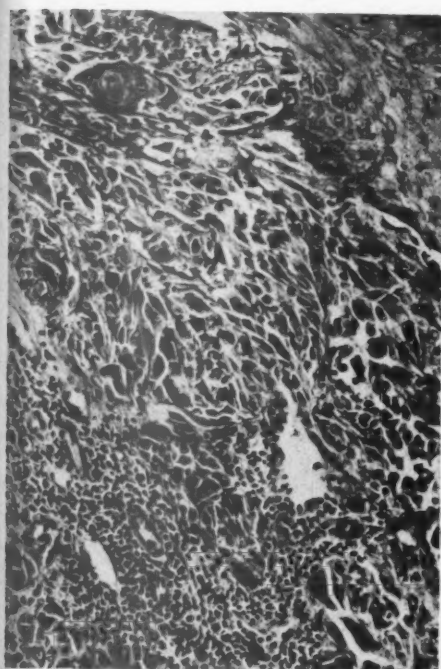
FIG. 11. Persistent giant cells in a melanoma of a female, 17 years old. Survival now 5 years. Hematoxylin and eosin stain. $\times 220$.

FIG. 12. Spindle cell structure in a fatal case of juvenile melanoma (female, 12 years old; death 4 months after local excision). Hematoxylin and eosin stain. $\times 180$.

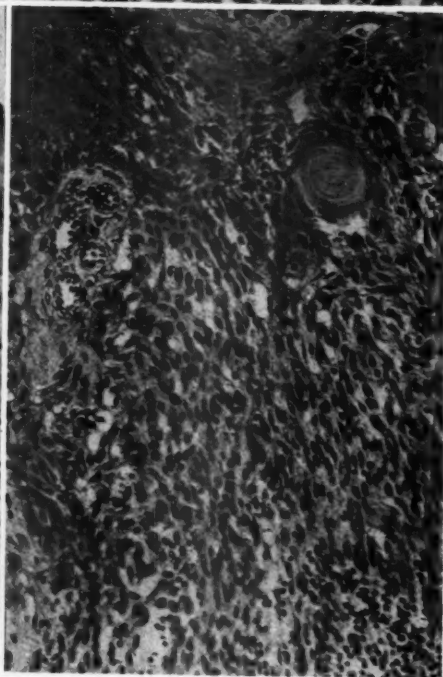
FIG. 13. Pleomorphic structure of a clinically malignant juvenile melanoma (case of Dr. Bjarne Pearson). Epidermis in this field has been destroyed by cautery. Hematoxylin and eosin stain. $\times 180$.

FIG. 14. Rapidly fatal malignant melanoma in a male, 14 years of age. Hematoxylin and eosin stain. $\times 160$.





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14

THE LESIONS OF SCHISTOSOMIASIS JAPONICA *

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During the early days of the occupation of the Philippine Islands in October and November, 1944, some of the American troops were unavoidably exposed to water infested with *Schistosoma japonicum*. The clinical picture of acute schistosomiasis japonica in this epidemic has been discussed by Thomas and Gage,¹ Billings *et al.*,² Winkler *et al.*,³ Tillman,⁴ and others. Description of the lesions of the acute stage has heretofore been lacking, except for depiction of human material by Ash and Spitz,⁵ and experimental observations in animals infested with *Schistosoma* reported by Fairley,⁶ Hoeppli,⁷ and Koppisch.⁸ Although death rarely occurs in the acute stage of schistosomiasis japonica, three of the soldiers exposed in the Philippine Islands died and were autopsied at overseas United States Army hospitals.⁹ Unfortunately, because of the local situation of the hospitals performing the post-mortem examinations, gross specimens were not preserved. Consequently, the complete picture in some of the organs is not available. In addition to material from the three cases studied at autopsy, tissue was secured for biopsy from acute lesions in the rectum, liver, and skin from other patients. Still later and unexpectedly, opportunity was afforded to secure material obtained for biopsy from cerebral lesions in American Army personnel who had returned to the United States. The older lesions of the disease, seen in three Filipinos who died in American Army hospitals following gunshot wounds, are included for comparison.†

The life history of *S. japonicum* has been described by Craig and Faust¹⁰ and more recently by Faust.¹¹ The ova are passed in the feces of infected man or domesticated animals and, on reaching fresh water, hatch within a few hours. The liberated miracidia penetrate the snail host (*Oncomelania quadrasi* in Leyte, P.I.) where first and

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† In preparation of this paper the following cases were studied: Army Institute of Pathology accession nos. 132699, 136057, 136056, 127286, 123276, 131466, 158928, 140914, 142999, 169042, 146469, 151096, 166305, 152989, 141886, 141575, 153128, 163485, 153127, 167247, 135996, 138331, 142999, 158928. Other aspects of nos. 141886, 141575, 138331, and 142999 have been reported by other authors elsewhere.

second generation sporocysts develop. After about 8 weeks cercariae emerge in great numbers. These adhere to the skin of man or animals at the level of the water surface and, as the skin dries, they burrow through the epidermis in the course of 8 to 10 minutes and enter a lymphatic vessel or venule. In this stage these larvae shed their tails and are known as metacercariae or schistosomulae. After traversing the systemic circulation for a few days, they collect in the small hepatic radicles of the portal veins where they mature in 4 weeks, mate, and for the next 5 to 15 years move around as pairs of adult flukes in the mesenteric veins, with the females depositing eggs in the smaller venules of the large and small intestine and appendix. A large number of ova are swept by the blood stream into the liver and a smaller number by way of the inferior vena cava to the lungs.

Ova also find their way to many other localities in the body. In the acute cases in this series, ova have been found in the mesenteric lymph nodes, skin, brain, meninges, and adrenal medulla. Ova have been demonstrated also in the late cases in retroperitoneal tissues, kidney, cerebellum, and medulla oblongata. In addition, lesions identical to those in which ova were demonstrated but in which the eggs were absent from the sections examined were present in the myocardium.

Although the lesions may vary depending on the structure of the tissue or organ involved, their similarity is to be emphasized. The early lesions are usually miliary, appearing as yellowish white, caseous nodules measuring from 0.5 to 10 mm. in diameter. Microscopically, in some of the lesions there is a necrotic zone around viable ova, as is shown in a section of a mesenteric lymph node (Fig. 1). This necrotic zone is surrounded by eosinophilic leukocytes and fewer neutrophilic leukocytes. The slightly older lesions present central ova, either viable or degenerated, with varying degrees of distortion and calcification, and surrounding epithelioid cells and fibroblastic proliferation in a richly vascular zone. Figure 2 demonstrates this stage in a lesion in the liver. Eosinophilic leukocytes decrease in number as the lesion progresses in age, and lymphocytes then predominate. Frequently the ovum is partially or completely surrounded by multinucleated giant cells. The latter are usually of the foreign body type, but they may have the appearance of giant cells of the Langhans' type, as seen in the brain in Figure 3.

The earliest lesions may coalesce to form large, irregular areas of necrosis in which are scattered the schistosome ova. This coalescent lesion is seen most often in the brain (Fig. 4), but it has been encountered also in the mesenteric lymph nodes. Frequently the early lesions

in the intestinal wall and liver assume the form of small abscesses containing the ova. It is not uncommon to find more than one ovum, even as many as ten, at the center of a lesion in the liver, mesenteric lymph nodes, or retroperitoneal connective tissue.

The only frequent reaction noted in the walls of blood vessels has been endothelial swelling in the capillaries, which may markedly narrow the lumina of the vessels. In the older lesions, perivascular cuffing by lymphocytes has been seen in the brain and liver. No lesion of the periarteritis nodosa type was seen in any of the cases.

Although the lesions produced by the ova of *S. japonicum* run a fairly characteristic course, they may vary depending on the organ involved. Those in the intestine occurred most frequently in the large bowel, but they were found also in the ileum in one early case, and in the ileum and duodenum in a late case. Johnson and Berry¹² have reported on the sigmoidoscopic picture in early cases. The miliary deposits of ova in the rectal mucosa gave the surface a coarsely granular appearance. Slight visible mucosal congestion occurred, but no ulcerations were seen by these investigators. Biopsies of the rectal wall in two patients in the early stages revealed ova in the mucosa and submucosa. In the biopsy of such a lesion the viable ova incite a granulomatous reaction (Fig. 5). A central necrotic zone may surround the ova, epithelioid cells are present, and the peripheral cellular reaction consists almost entirely of eosinophilic leukocytes. In the later cases the lesions in the intestine show a moderate amount of fibrosis, and the eosinophils are replaced by lymphocytes. One section of the duodenum of a late case demonstrated ova passing through the mucosal epithelium to enter the lumen of the bowel (Fig. 6). The appendix was involved in several cases and showed a reaction similar to that seen in the rest of the intestine.

The lesions in the liver (Fig. 2) follow the pattern seen in the intestine. However, as the disease progresses, the compact fibrosis which is so characteristic of the later stages spreads along the portal radicles to produce the picture of "pipestem" cirrhosis described by Stitt¹³ in his textbook on tropical diseases. In the early stages, endothelial swelling has been seen in the central veins of the lobules, and portal thrombosis was noted only in one case. Late cases showed hemosiderin-like pigment in the Kupffer cells.

The lesions in the lungs tend to remain discrete rather than to become confluent, and serial sections of late lesions showed that they remain as nodules rather than developing fibrosis along the course of the pulmonary vessels. However, the lung of one case in the acute

stage showed an interstitial pneumonitis. Numerous eosinophils were present throughout the sections. A few ova were demonstrated in the sections of this lung.

Mesenteric lymph nodes of acute cases revealed hyperplasia of the germinal centers of the follicles. The sinusoids were filled with lymphocytes, plasma cells, and a few eosinophilic leukocytes. In one acute case showing ova in the lymph nodes, the ova were engulfed by giant cells of the Langhans' type and the surrounding tissue was necrotic. In advanced lesions the ova were present in the center of dense fibrous tissue nodules. Pigment similar to that seen in the liver was present in a relatively small number of reticulo-endothelial cells in the late cases.

Although the intestine, liver, and lungs are the most frequent sites of involvement, other organs may be involved as has been noted previously. Several patients who had returned to the United States were later operated upon because of various symptoms of disease of the central nervous system, and biopsy of the cerebral lesions revealed ova of *S. japonicum*. Vitug *et al.*,¹⁴ Carroll,¹⁵ Tillman,⁴ and others have reported on the cerebral involvement in this disease. In these diffuse lesions a soggy cortex and medulla were produced. Small discrete lesions frequently were not recognizable grossly. Microscopically, necrosis was widespread (Fig. 4). Eosinophils were present in moderate numbers, but small round cells, including both lymphocytes and plasma cells, sometimes predominated at the periphery of the necrotic zone. Swelling of the capillary endothelium in the region of the lesions was a fairly constant finding. Perivascular cuffing by lymphocytes occurred in the late cases. The acute and advanced lesions otherwise resemble the classical "pseudotubercle" seen in other organs. In the relatively early cases ova have been found in the cerebral medulla of the temporal lobe, middle and posterior superior temporal gyri, parietal lobe, and right occipital lobe. Examination of a late case disclosed ova in the medulla and in the cerebellar cortex.

Biopsy of a nodule in the skin of the abdominal wall, one of a chain of such nodules following the course of an intercostal blood vessel (described by Fishbon¹⁶), revealed the acute lesion of schistosomiasis japonica, with a centrally placed ovum. An arteriole in the section showed focal endothelial swelling. The vessel was surrounded by hemorrhage.

A very extensive myomalacia cordis was present in the left ventricle in one acute case of schistosomiasis. Fibrinous pericarditis overlaid the involved myocardium and there was a large mural thrombus. Sec-

tions showed widespread necrosis and degeneration of myocardial fibers. There was marked fibroblastic proliferation and cellular infiltration of plasma cells and lymphocytes, with fewer neutrophilic and eosinophilic leukocytes. Arterioles and venules in the degenerated area presented hyalinoid necrosis without cellular infiltration of their walls. There was early organization of the inflamed pericardium. The mural thrombus contained many leukocytes of which the greater number were eosinophils, but was without organization. The cause of the infarction was not apparent, for ova were not demonstrated in the heart or pericardium. The coronary blood vessels were not available for further study. In a late case showing myocardial involvement, the lesion was localized to a small area heavily infiltrated by lymphocytes. At the center of the lesion there was a foreign body giant cell. The ovum which undoubtedly produced the lesion was not seen in the several sections examined.

One of the acute cases disclosed ova in the adrenal medulla. An eosinophilic cellular infiltration surrounded them.

A late case presented a focal area of necrosis in the pituitary gland, but ova and cellular reaction were absent.

The genitourinary system showed nothing of note in the acute cases. In one late case an ovum was found in the afferent arteriole of a glomerulus. Slight lymphocytic cellular infiltration had occurred around this glomerulus.

Passive congestion and slight enlargement were the only notable findings in the spleen in the acute cases. In the late cases pigment similar to that seen in the Kupffer cells in the liver appeared in the reticulo-endothelial cells. Of the three late cases the largest spleen weighed 340 gm. and the smallest, 135 gm.

Extreme fibrosis of retroperitoneal tissues was present in one late case, a female with unilateral edema in the left leg. Ova of *S. japonicum* were present singly and in clusters in the fibrous tissue. The latter was relatively vascular and lightly infiltrated by lymphocytes.

SUMMARY

In tracing the lesions of schistosomiasis japonica from the early to the advanced stages, the disease in man is seen to bear a marked similarity to the lesions of schistosomiasis produced experimentally in animals. The general appearance of the lesion varies somewhat, depending on the organ involved, but as a rule it follows a fairly uniform course. When the ovum enters the tissues an extensive cellular reaction occurs, and this consists chiefly of eosinophilic leukocytes, with

fewer neutrophilic leukocytes. In some of the lesions necrosis of tissue occurs in a fairly wide zone around the ovum. Epithelioid cells appear and multinucleated giant cells engulf the ovum. The inflammatory cellular response changes to one in which lymphocytes and plasma cells are most numerous. Fibroblastic and capillary proliferation begin early in the peripheral zone, and, as the lesion advances in age, fibrosis predominates. The oldest lesions consist of shrunken, calcified ova surrounded by more or less dense fibrous tissue, with moderate lymphocytic cellular infiltration. In this pathologic picture of the disease the early lesions represent an unusual and characteristic reaction to the viable ovum with necrosis and eosinophils, and the later lesions represent a foreign body reaction.

We gratefully acknowledge the cooperation of the Army Institute of Pathology which furnished much of the material for examination and permitted the use of the photomicrographs reproduced with this paper.

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[Illustrations follow]

DESCRIPTION OF PLATES

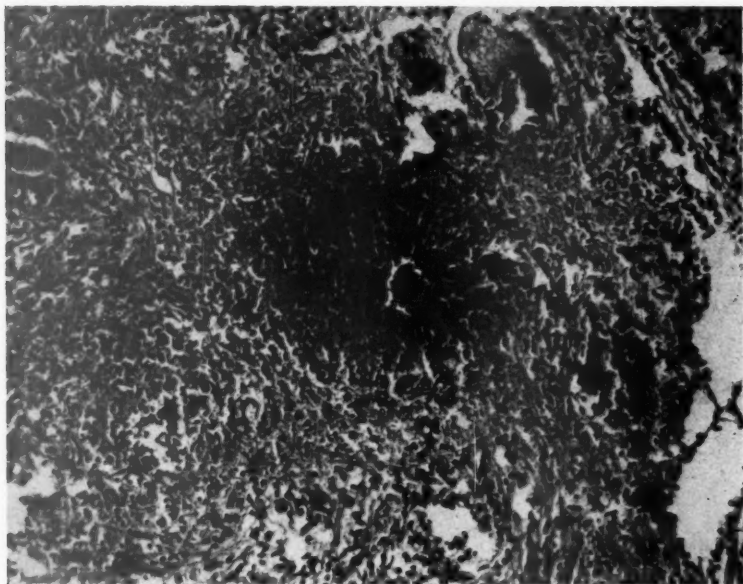
PLATE III

FIG. 1. Necrotic zone surrounding an ovum in a lymph node. $\times 145$. (Army Institute of Pathology negative no. 97398.)

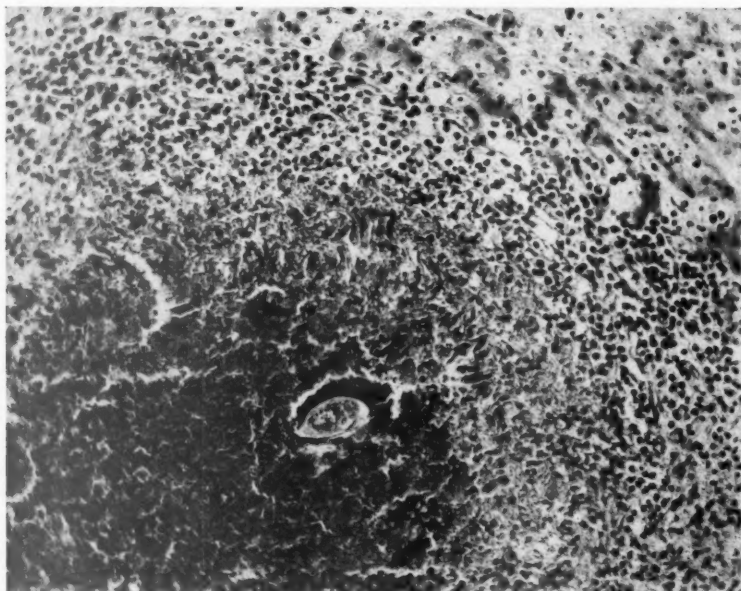
FIG. 2. Viable ovum, surrounded by necrosis and numerous eosinophils in the liver. $\times 175$. (A.I.P. neg. 97396.)



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Bracken, Bailey, and Thomas

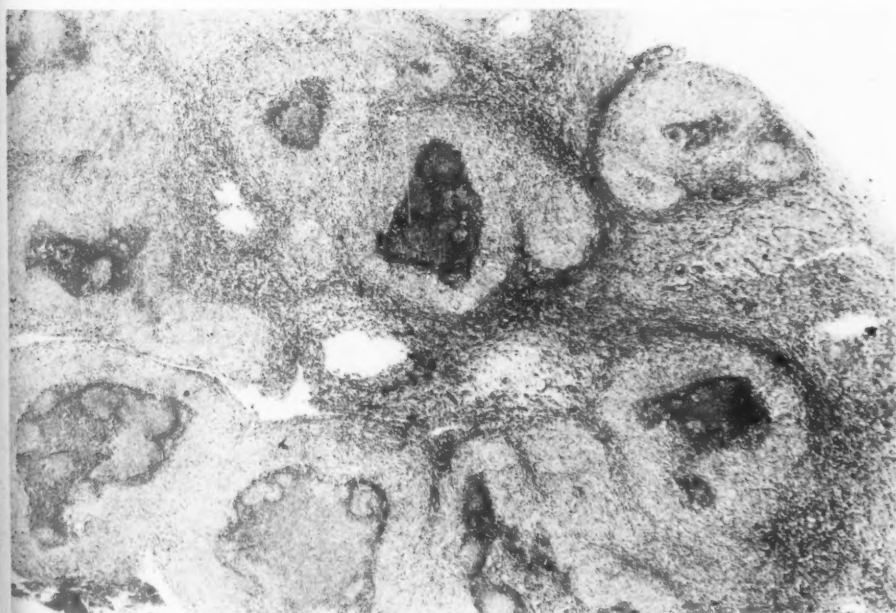
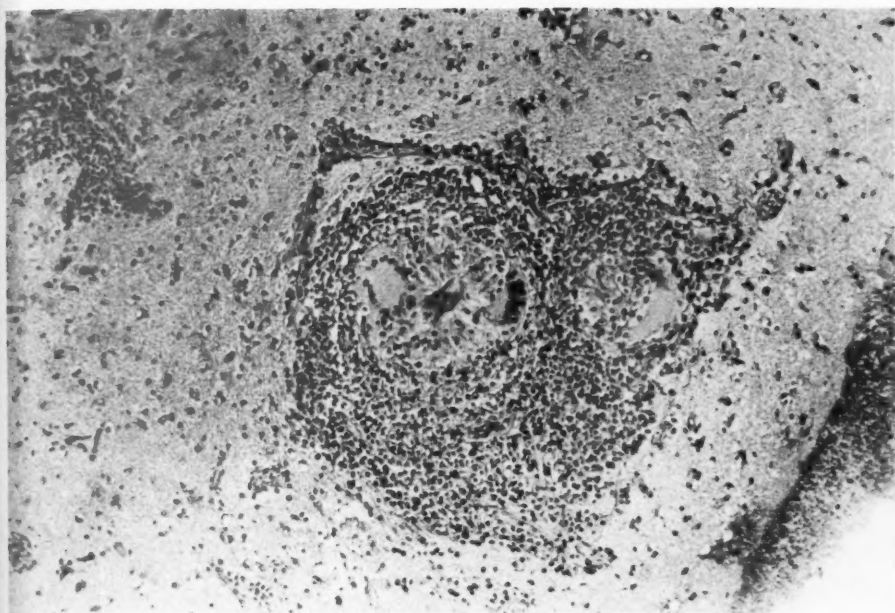
Schistosomiasis Japonica

PLATE 112

FIG. 3. Lesion in cerebral medulla, with one distorted ovum, multinucleated giant cells, epithelioid cells, and leukocytes. $\times 120$. (A.I.P. neg. 93480.)

FIG. 4. Coalescing lesions in the brain. $\times 20$. (A.I.P. neg. 43469.)





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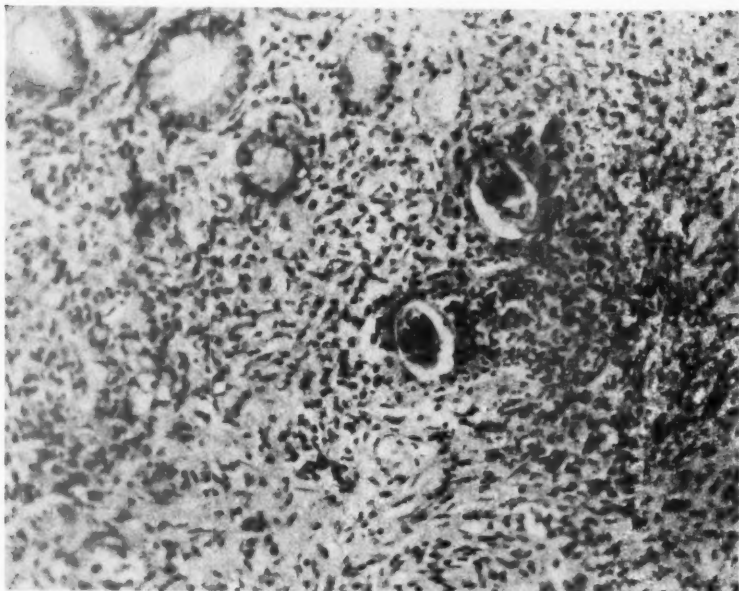
Schistosomiasis Japonica

PLATE 113

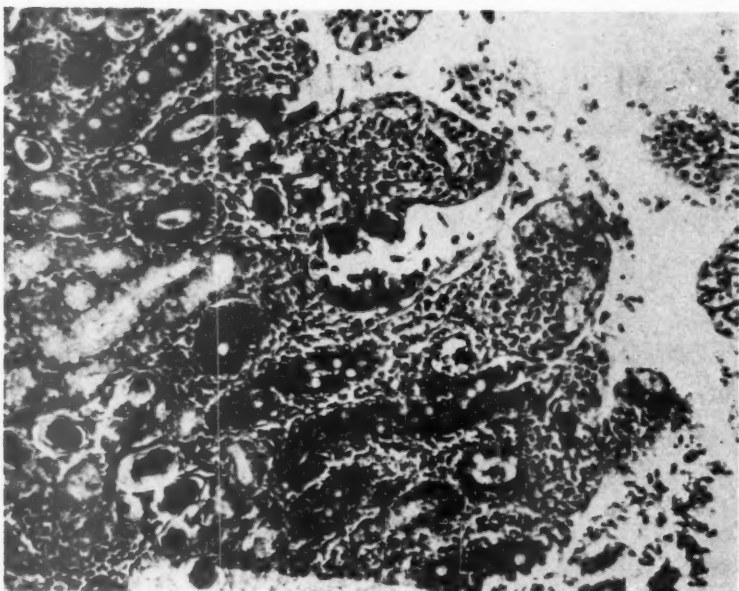
FIG. 5. Viable ova in rectal mucosa. $\times 210$. (A.I.P. neg. 97389.)

FIG. 6. Ova being extruded into the lumen of the duodenum. $\times 145$. (A.I.P. neg. 97385.)

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THE SIGNIFICANCE OF LOCAL VASCULAR PHENOMENA IN THE PRODUCTION OF ISCHEMIC NECROSIS IN SKELETAL MUSCLE *

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The occurrence of a peculiar form of degeneration induced by ischemia in skeletal muscle has been described in a previous publication.¹ It was considered to be identical with Bowman's discoid change, which consists of transverse fragmentation of skeletal muscle fibers into broad anisotropic disks. The degeneration is an invariable result of prolonged, unrelieved ischemia, and has been conclusively associated with the pathogenesis of ischemic necrosis of muscle by several observers, both experimentally¹⁻³ and clinically.^{4,5} From experimental studies it is apparent that the disks first appeared subsequent to unrelieved ischemia of 4 hours' duration, and thereafter increased until they were nearly ubiquitous at 18 hours. Since all fibers so affected were non-viable,¹ their rate of development was considered to be a satisfactory index of the rate of necrosis of the fibers. It was further found that release of the tourniquet after 4 hours of ischemia did not preclude extension of discoid degeneration, which was as considerable after 18 hours as that associated with a similar period of unrelieved ischemia. Because of this observation it is apposite to inquire more extensively into the vascular phenomena associated with the pathogenesis of acute and chronic ischemic necrosis of skeletal muscle. Although occlusion of major vessels may initiate the process, other factors appear to operate toward perpetuation of the ischemia when the external pressure is relieved.

The rôle played by the vessels in the pathogenesis of ischemic necrosis of skeletal muscle was first evaluated in the classical clinical descriptions by Volkmann⁴ and his pupil, Leser,² who ascribed the necrosis of muscle to the ischemia induced by compression of the arteries with constrictive dressings. However, the steady accumulation of cases in which dressings could not be implicated unsettled this view, which was finally replaced by the hypothesis that necrosis was caused by venous obstruction due to a local hematoma,⁶ an opinion shared by Brooks from experimental studies.⁷ This assumption remained undisturbed until it was clearly shown by Griffiths⁸ that the lesions caused by venous and arterial occlusion are distinct, and that

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the former in no way resemble those of ischemic necrosis. On the basis of his experience with arterial embolism and direct trauma to arteries, he advanced the opinion that the ischemia was due to arterial spasm, a point of view supported by Barnes and Trueta⁹ and Leriche,¹⁰ so that culpability came to rest again upon the arteries.

It is noteworthy, however, that no consideration had been given to the capillaries. Except for the work of Wertheimer, Dechaume, and Frieh,¹¹ who plugged the venules by retrograde injection of a gelatin mass, which is virtually capillary obstruction, no study of these vessels in relation to ischemic necrosis has been uncovered. Yet any assessment of the vascular phenomena peculiar to ischemia must take the intimate vasculature into consideration. This is particularly necessary in skeletal muscle because of the unique gradient of permeability in muscle capillaries¹² and the consequences which disturbances of the gradient may produce in the tissue.

METHOD

Complete ischemia was produced in the right hind legs of albino male rabbits weighing 2,000 to 3,000 gm. The animals, which were permitted food and water freely, were first narcotized by an intraperitoneal injection of 35 mg. of "veterinary" nembutal per kg., usually in a volume of 5 cc. Ischemia was then induced in the right hind leg by the application of tourniquets, such as were used for rats by LePage,¹³ or by the use of a tightly wound rubber band (Eberhard Faber no. 64), which was applied according to the method of Rosenthal.¹⁴ These were adjusted so as to encircle the thigh about 0.5 cm. proximal to the knee, because with higher obstruction the animals frequently died in shock. Complete arterial occlusion was confirmed by lack of swelling in the ischemic leg as well as by the failure of fluorescein to pass distal to the tourniquet when it was injected intravenously in amounts as great as 5.0 cc., and the leg was inspected under an ultraviolet lamp screened to exclude all except light of 4,000 Å.¹⁵ Preference was given to the rubber band method as a means of producing ischemia because of the instantaneity of application and release which it permitted. The obstruction was removed at the end of the period of ischemia. The great saphenous artery was sought by palpation, and its pulsation identified before further procedures were instituted. It could be rolled against the tibia and felt to pulsate vigorously.

Histologic studies were made on formalin-fixed tissue, prepared as paraffin sections, cut to a thickness of 7 μ , and stained with hematoxylin and eosin. Although a more extensive histologic analysis is in prep-

aration, the general histologic features of the diseased tissues will be described, in so far as they have a bearing upon the problem immediately under investigation.

EXPERIMENTAL PROCEDURES

Investigation of Large Arteries and Veins

Angiography was carried out on animals subjected to ischemia for from 4 to 4½ hours with a release of obstruction for 3, 24, and 48 hours thereafter. Each animal was anesthetized with nembutal and, when required, with ether. A laparotomy was performed and the abdominal aorta was dissected free of its surrounding tissue. With the animal under the x-ray apparatus, a 20 gauge needle was inserted into the vessel and thorotrast (Heyden Chemical Co., 24 to 26 per cent ThO₂ by volume) slowly injected without interference with blood flow. After 5.0 cc. had been injected, an exposure was made (factors 50 ma, 50 mv, at 36 inches distance and for ¾ second). When a further final 5.0 cc. had been introduced, a second exposure was made, and a third made 1 minute subsequent to completion of the injection. In several animals sodium iodide, made up as a 20 per cent solution in isotonic sodium chloride, was used, but because of the violent muscle spasm, hyperpnea, and convulsions attendant upon its injection it was not used routinely.

In a few animals a 20 per cent (by volume) solution of Higgins' India ink was injected in a similar manner to that employed for thorotrast, in amounts equivalent to 3.0 cc. per kg. The needle was held in the aorta so that blood flow was not impeded during infusion; thus the injections were comparable to those with thorotrast. Within a few minutes the animal was sacrificed and both extensor and flexor muscles excised from the ischemic and normal legs. Tissue from both groups was then fixed in 10 per cent formalin and subsequently cleared by the method of Spalteholz, to permit direct visualization of the injected vessels.

Irrespective of the duration of the ischemia, it was possible immediately after release of the tourniquet to palpate the pulse in the great saphenous artery. Subsequently, with the accumulation of edema fluid, it was often difficult to detect the pulse unless the fluid was well pressed aside digitally, when it was usually readily accessible. In the few instances when palpation was equivocal, the skin was reflected and pulsation determined by direct visualization. The angiographic studies in 10 instances (Table I) confirmed this patency, and are worthy of further interpretation. The first roentgenogram (Fig. 1) is an example

of a first exposure, in which it is seen that not only were the arteries patent and well filled, but that those on the right were more dilated than the normal vessels. In Figure 2, which represents the second exposure, the veins had begun to fill on the normal side whereas those on the ischemic side were not visualized. In the third roentgenogram (Fig. 3) the venous filling on the left or normal side was less, and that on the right more apparent. This sequence was repeated in all 10 cases. From this it may be inferred that the arteries and veins were patent, but that there was a slowing of the circulation in the smaller vessels of the tissues between the arteries and veins. There was no especial indication that the thorotrast passed through the muscles, for

TABLE I
Angiography (by Thorotrast) of Limb Ischemia (4½ Hours)

| Number of experiments | Interval between release of tourniquet and x-ray | Radio-opacity of vessels | | Pulse* |
|-----------------------|--|--------------------------|-------|--------|
| | | Arteries | Veins | |
| 5 | hours 1½-4 | + | + | + |
| 3 | 24 | + | + | + |
| 2 | 48 | + | + | + |

* Pulse determined by palpation of great saphenous artery.

Factors in roentgenography were: 50 kv, 50 ma, ¼ second, and 36 inches.

it might well have circumvented the muscles by shunting through the skin and subcutaneous tissue. However, in the India ink preparations (Fig. 4) particles were observed in the small intramuscular arteries, although the finer ramifications were not so conspicuous as in the contralateral normal muscles. The filling of these small vessels within the tissue indicates that the thorotrast may take a similar path, since its particles are comparable in size to those of the ink.

Investigation of Penetration of Dye

Bromphenol blue (tetrabromophenolsulfonphthalein) was the dye used in studies of the rate of penetration into, and elimination from, ischemic muscle of small, diffusible molecules. It was first used in biologic research by Rous and Drury¹⁶ in their investigations upon graded permeability of fine vessels in rabbit skin. They used a 4 per cent solution, but a 2 per cent solution was used here and injected into the right ear vein in amounts equivalent to 3.0 cc. per kg. The animals so injected were divided into two groups. In one series the dye was

injected within 5 minutes of the release of the tourniquet. Animals were then selected at intervals of $\frac{1}{2}$, 3, and 20 hours, anesthetized with nembutal, and the skin of both hind legs reflected so that direct inspection of the muscles was feasible. The intensities of impregnation of the dye in the ischemic and normal muscles were compared and classified as 0 to 4 plus. In the other group of animals injection of the dye was delayed for 20 hours, after which they were treated in a manner similar to those of the first group. In both groups, when the in-

TABLE II
Passage of Bromphenol Blue (2 Per Cent) through Muscles

| Immediate dye injection (5 min.)* | | | |
|-----------------------------------|---------------------------|---------------|-----------------|
| Ischemia | Before observation | Normal muscle | Ischemic muscle |
| <i>hours</i> | <i>hours</i> | | |
| 2 | 3 $3\frac{1}{4}$ 20 | 0 0 0 | \pm 0 0 |
| 3 | $\frac{1}{2}$ 3 20 | 3+ 0 0 | 4+ 2+ 0 |
| 4 | $\frac{1}{2}$ 2 20 | 3+ 0 0 | 4+ 3+ 1+ |
| 6 | $2\frac{1}{4}$ 20 | 0 0 | 2+ 1+ |
| 8 | 18 20 | 0 0 | 2+ 1+ |

* The dye was injected within 5 minutes after release of the obstruction.

tensity of the dye was judged, the tibialis anticus and extensor digitorum longus muscles were peeled off the anterior aspect of the leg as a group and the plantares muscles excised from the flexor mass.

The injection of bromphenol blue (Table II) immediately subsequent to release of the tourniquet was followed by a rapid intense staining of both normal and ischemic muscles, indicating lack of arterial occlusion and free penetration of dye regardless of the duration of ischemia. When an interval of 3 hours elapsed between the injection of dye and the determination of intensity of staining, a distinct pattern of behavior became apparent. Dye was either eliminated entirely or was only scantily retained by muscles which were ischemic for 2 hours; when elimination was retarded, considerable dye was demonstrable in the plasma, indicating that excretion of dye by the

animal was not rapid. When ischemia had been of 3 or more hours' duration, there was considerable retention of dye by the muscle even 3 hours after injection. Consideration of the fate of the dye 20 hours after its infusion revealed that, with such a time interval, evidence of sluggish elimination was first manifest in muscles ischemic for 4 hours. From these findings it may be seen that circulation through the muscle was extremely sluggish. It was impossible to determine whether

TABLE III
Passage of Bromphenol Blue (2 Per Cent) through Muscles

| Delayed dye injection (20 hr.)* | | | |
|---------------------------------|--------------------------|---------------|-----------------|
| Ischemia | Before observation | Normal muscle | Ischemic muscle |
| <i>hours</i> | <i>hours</i> | | |
| 2 | $\frac{1}{2}$ 3 20 | 2+ o o | 3+ ± o |
| 3 | $\frac{1}{4}$ 3 20 | 3+ ± o | 3+ 1+ o |
| 4 | $\frac{1}{4}$ 3 20 | 3+ o o | o 2+ ± |
| 6 | $\frac{1}{2}$ 4 20 | 3+ o o | o 2+ ± |
| 8 | $\frac{1}{4}$ 1 3½ | 3+ 3+ ± | o o 2+ |

* Dye was injected 20 hours subsequent to release of the obstruction.

this slow intramuscular circulation was due to impairment of flow within the muscle or to deviation of blood to other structures; the fact that the dye was eliminated from the skin more rapidly than from muscle would appear to favor the latter view, whereas it might signify merely a relatively greater resistance of skin vessels to ischemia as compared with muscle vessels.

When the injection of dye was deferred until 20 hours had elapsed after release of the tourniquet, the behavior of the dye differed considerably from the preceding pattern (Table III). With ischemia of 2 and 3 hours, respectively, the dye entered quickly and was rapidly eliminated; where elimination appeared retarded, it was found that the plasma was still laden with dye and that the normal muscle had still retained a trace, so that the apparent deficiency of elimination was not significant. After ischemia of 4 to 6 hours' duration, a striking de-

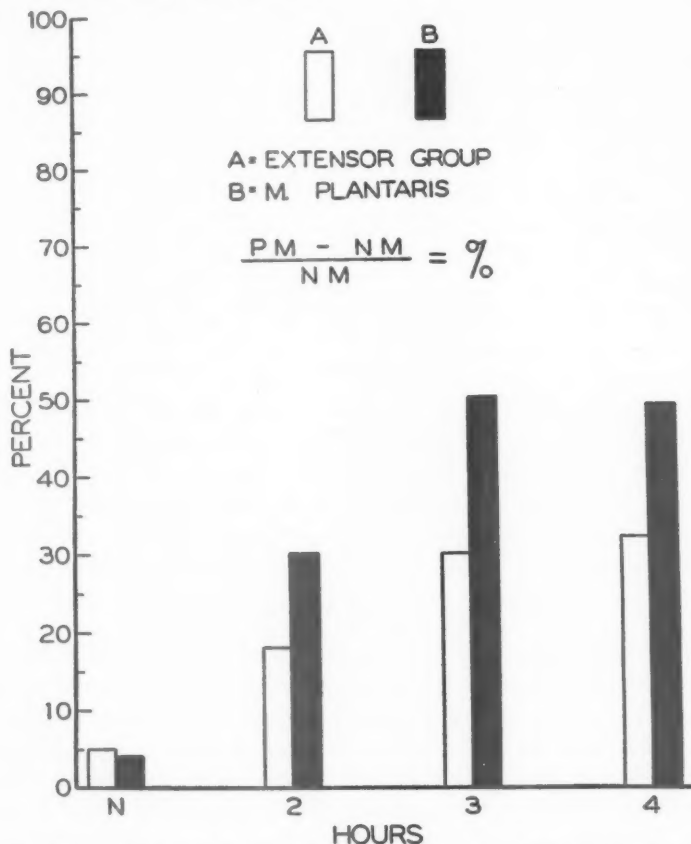
parture was observed in that the dye failed to enter the ischemic muscle for at least $\frac{1}{2}$ hour subsequent to injection, whereas the contralateral muscles were stained to their usual intensity. But 3 hours later the abnormal muscles were deeply stained, when the normal muscle had entirely lost its coloration, so that the circulation was still open, although very slow. In the muscles ischemic for 8 hours no dye had entered even after 1 hour, yet penetration was considerable in a little over 3 hours. It was apparent that with ischemia of 4 hours or longer, and despite a return of blood flow, the circulation re-established through the muscle was abnormally sluggish. On the other hand there was never any delay in staining of the skin comparable to that seen in the muscle.

The histologic picture of the muscles several hours after release of vascular obstruction was complicated by a profuse infiltration of monocytes and polymorphonuclear leukocytes, considerable edema, and extensive degeneration of the muscle fibers. Many fibers were split up into Bowman's disks, some were fragmented into minute, irregular, granular, basophilic particles, and a few had a pale, eosinophilic, waxy appearance; of the several forms of degeneration the Bowman type was predominant (Fig. 5). In these muscles the capillaries appeared ubiquitous, dilated, and engorged with tightly packed erythrocytes, which appeared square because of the pressure due to mutual contiguity. They contained no thrombi or cell-free segments, and extravasations from them were rare and minute. No alterations were observed in the structure or contents of the larger arterial and venous channels.

Assessment of Local Fluid Accumulation

The excised groups of muscles, both ischemic and normal, were in each instance stripped of fascia, pressed dry between filter papers, and weighed rapidly to the nearest 0.01 gm. In a series of normal animals, the difference in weight between the extensor group and the plantaris of the right leg and these muscles in the left was determined to ascertain the normal variation, using the left leg muscles as the standard, and expressing the difference in weights as a percentage of these muscles. Since the right leg usually was rendered ischemic, the increase in weight of its muscles was also expressed as a percentage of the left in a similar manner. It was expected that this would adequately indicate any trend or significant deviation in weight as readily as comparing differences in ratios of wet and dry weight, since it would also be necessary to assume in the latter method that the loss of necrotic tissue by flushing out was balanced by plasma proteins brought in with the edema fluid. It has been stated by MacFarlane and Spooner¹⁷

that the maximum increase in weight due to edema is reached within $\frac{1}{2}$ hour for guinea-pigs, and by Koletsky and Gustafson¹⁸ that it is reached in 3 hours in rats after release of ischemic obstruction to a limb. Since the ischemic muscles weighed in this series were all released for 3 hours or longer, it may be assumed that all had attained their maximum degree of edema.



Text-Figure 1. The duration of ischemia is plotted against percentage increase in weight. PM = weight of ischemic muscle. NM = weight of normal muscle. Each column represents the average of ten or more estimations. Column N signifies the average maximum increase between corresponding normal muscle in a series of untreated controls.

The average percentile difference in weight between similar muscles in the leg, those in the left leg being used as standard, is plus or minus 3 per cent. The average weight difference of the ischemic muscles expressed in a similar manner is demonstrated in Text-Figure 1; in

which the increase falls far outside the normal range of variation between similar muscle groups. It was striking that there was considerable edema after even 2 hours of ischemia, but that the difference between this and the edema following 3 hours of ischemia was marked. On the other hand, the edema after 3 and 4 hours of ischemia was not significantly different. It was further apparent that the extent of edema in the plantaris muscle, a representative of the flexor group, was invariably much greater than that of the extensor group as represented by the tibialis anticus and extensor digitorum longus, a fact which must be accounted for in determining the importance of edema *per se* in abetting the effects of ischemia.

Development of Chronic Lesions

In a further set of experiments, the animal was not disturbed after release of the tourniquet, except to examine the pulse and estimate the extent of edema and paralysis, until a period of 10 to 30 days had

TABLE IV
Relation of Duration of Ischemia to Development of Chronic Lesions

| Number of Experiments | Ischemia | Duration of experiment | Infarcts | Average change of weight* |
|-----------------------|--------------|------------------------|----------|---------------------------|
| | <i>hours</i> | <i>days</i> | | |
| 4 | 3 | 10-30 | 0 | -30% |
| 5 | 4 | 10-24 | 2 | -30% (No infarct) |
| | | | | +50% (Infarcted) |
| 4 | 6 | 12-15 | 4 | +50% |
| 2 | 8 | 15-16 | 2 | +30% |
| 2 | 12 | 10-18 | 2 | +80% |

* All muscles which increased in weight contained infarcts; those without infarcts were decreased in weight.

elapsed. At the elected time the animal was anesthetized and injected with 4 per cent bromphenol blue to stain the tissues intensely. The leg muscles were then exposed after $\frac{1}{2}$ hour, a period adequate to allow staining of normal tissue. The presence of gross areas of necrosis was determined in the muscles; such areas were avascular and consequently free of dye. The muscles were then excised and treated similarly to the previous groups.

In the light of the previous findings, the late sequelae of varied periods of ischemia of skeletal muscle were very significant (Table IV). The muscles which were ischemic for less than 4 hours were shrunken and firm, elastic and contractile, and stained very deeply with the injected dye (Fig. 6). When the difference in weight was

expressed as a percentage of the weight of the normal muscle, it was found that the weight of the ischemic muscle was significantly decreased, although no gross lesions were observed. When ischemia had lasted for 4 hours, some muscles resembled those subjected to 3 hours of ischemia; others contained large, greenish yellow, depressed, hard, friable areas, sharply demarcated from the ends of the muscle (Fig. 7) and the surrounding normal tissue. The yellow areas were not contractile, although the parts surrounding them were electrically irritable. Such areas of necrosis occurred in all tibialis anticus and plantaris muscles ischemic for longer than 4 hours, and less commonly involved the gastrocnemii. They tended to include the whole muscle except for a small portion adjacent to the tendinous attachments. It was noteworthy that the weights were much increased in those muscles which contained infarcts.

Microscopically, the infarcted areas were composed entirely of fibers which were segmented into anisotropic Bowman's disks or conchoidal plates and which contained no nuclei (Fig. 8). The disks were not seen in the surrounding normal muscle, which was separated from the infarct by a zone of richly vascular, fibroblastic connective tissue in which there were occasional multinucleated muscle cells. The most conspicuous result from this series was that it indicated the occurrence or completion of some phenomenon at the end of 4 hours of ischemia, because of which the character of the final muscle damage was altered from a diffuse moderate wasting caused by the shorter periods to a severe massive necrosis with impairment of function.

DISCUSSION

From the histologic character of the areas of infarction or "muscle sequestra," as Griffiths⁸ termed them, the lesions caused by temporary arterial occlusion are identifiable with those found in experimental ischemic necrosis due to unrelieved arterial occlusion.^{1,3} This lesion is comprised of a central mass of yellow necrotic tissue, made up of fibers segmented into Bowman's disks, around which is a zone of fibroblastic connective tissue, the vascularity of which is considerable. This differs from the lesion caused by venous occlusion, in which there is no sequestrum, but merely a sparse scattering of atrophic muscle fibers widely separated by dense fibrous tissue,¹⁹ a picture never seen with arterial occlusion. Furthermore, the experimental lesions are identical with those found in Volkmann's contracture. From this histologic evidence it is possible to infer that venous obstruction plays no part in the pathogenesis of experimental and clinical ischemic necrosis of

skeletal muscle; this is substantiated by the free venous filling observed in the roentgenograms.

The involvement of the large arteries also may be excluded because of the invariably palpable pulse along the great saphenous artery, which is an index of the patency of large arteries below the site of obstruction. The reliability of this criterion as an index of adequate arterial patency is dependent upon the finding of Mann *et al.*,²⁰ that it is necessary to reduce the lumen of a vessel to 12 per cent of its normal area before the blood flow to the area beyond the obstruction is nearly halved. In all instances the easy palpation of the pulse indicated an expansion of the arterial lumen compatible with normal flow, since it is known that even after 50 per cent reduction in size of the lumen there is no appreciable effect upon rate of flow. This patency is amply confirmed by the angiographic studies which revealed not only patency but an actual dilatation of the arterial tree in the distal part of the previously occluded limb. The discrepancy of this finding with those of Barnes and Trueta⁹ may be due to the difference in radio-opaque medium used. They employed a solution of sodium iodide, which was highly irritating and which induced severe muscle spasm. I had similar experience with it, and adopted the use of thorotrast, which is non-irritant and has a neutral reaction. With the latter medium, spasm was never encountered. While these experiments cannot exculpate arterial spasm entirely as an occasional cause of muscle necrosis, they exclude it as the common mechanism arising from temporary arterial occlusion, such as Griffiths,⁸ Barnes and Trueta,⁹ and Leriche¹⁰ suggest.

While it is irrefutable that arterial occlusion initiates the process of ischemic necrosis, it is equally certain that some other cause than persistent arterial obstruction must be sought to explain the perpetuation of the damage so inaugurated. The behavior of bromphenol blue in its slow penetration into, and tardy elimination from, the muscle indicates that the fault lies within the muscle itself. That this behavior of the dye is caused by adsorption to the proteins of inflammatory exudate appears improbable because it has been shown by Miller²¹ under different experimental conditions that this particular dye was absorbed more rapidly from inflamed than from normal tissue. The slow entrance of the dye would favor the contention, however, that the blood is shunted away from the muscle and into the skin, which is usually rapidly tinted. The mechanism of this would be by sympathetic paralysis, which, as Siddons²² and Cohen²³ pointed out, causes a deviation of blood flow into the skin at the expense of the muscles. And it must

be recalled that ischemic contracture is almost invariably accompanied by nerve injury.²⁴ Consequently, it is not possible to discount entirely this variation in circulation on the present evidence. Caution is further implemented by the finding that in man, intra-arterial adrenalin infusion causes vasodilatation in the muscles of the leg,²⁵ followed by vasoconstriction, independently of sympathetic action.

On the other hand, this is not the decisive factor because a profound paralysis is induced by ischemia of 2 and 3 hours, after which infarction always has been absent. This being so, it is probable that the cause of the sluggish flow through the damaged muscle lies within the tissue and is of such a nature as to impede the inflow of arterial blood. Since the veins are not blocked, the site of the obstacle must be in or around the venules, capillaries, and fine arterioles. Histologically, these minute vessels are silted up with red cells, which signifies that the liquid component of the blood has filtered off almost entirely and left behind a vessel plugged with conglutinated particulate elements. This is the picture of stasis invoked by severe capillary damage.^{26,27} Because of this capillary damage, stasis, and the obliteration of the normal gradient of permeability,¹² there is a considerable resistance to the inflow of blood, and considerable depression of the rate of exchange of metabolites, of which bromphenol blue may be a reliable criterion since its diffusion coefficient is not far removed from that of dextrose. The difference in the degree and type of muscle damage caused by periods of ischemia of less than 4 hours as compared with those of longer periods is perhaps to be explained by a peculiar vulnerability of the fine vessels to different durations of ischemia, for, though damage apparently follows the shorter periods of ischemia as indicated by edema, it may be irreversible only after the longer. It must be remembered also that, although considerable interstitial fluid accumulates after release of occlusion, judged by weight this is as great after 3 as after 4 hours of ischemia, so that if diffusion through the increased volume of fluid is invoked as a factor in the impairment of metabolic exchange,¹⁹ it could not explain the difference in reversibility between these two periods. Furthermore, the increase in weight of the plantaris muscle is as great after 2 hours as is the increase of the extensor group after 3 or 4 hours of ischemia, although infarction does not appear in the plantaris until after 4 hours of ischemia. Therefore, one would suspect this difference to be due to direct damage to the capillary wall by the ischemia.

It is pertinent to draw attention to the studies by Meneely and co-workers²⁸ on the results of temporary occlusion of the coronary artery in a Bailey-LaDue preparation, which demonstrates progressive dam-

age to the myocardium, as revealed by electrocardiography, after occlusion is released and blood flow re-established. They believed that this is the result of increased capillary permeability to trypan blue. Their conclusion concerning the pathogenesis of ischemic necrosis in cardiac muscle is essentially similar to that which I have reached on the basis of the studies outlined above.

SUMMARY

1. By means of direct palpation and visualization of pulsation and with supplementary angiography, spasm of arteries is excluded as a major factor in the pathogenesis of ischemic necrosis of skeletal muscle.
2. The angiographic studies and histologic lesions indicate that the condition is not caused by venous obstruction.
3. In view of the manner of movement of the dye bromphenol blue into and out of ischemic muscles and the histologic picture of stasis it is most likely that the principal cause of the ischemic necrosis is the persistence of initial ischemic damage of the intimate vasculature.

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DESCRIPTION OF PLATES

Plate 114

- FIG. 1. Thorotrast injection in an animal with ischemia of the right leg for $4\frac{1}{2}$ hours, and subsequent release for 4 hours prior to injection. The roentgenograph was taken when 5 cc. of thorotrast had been injected into the aorta. The arteries of both legs are clearly visualized; there is dilatation of those in the right leg below the site of ischemic obstruction. $\times \frac{1}{4}$.
- FIG. 2. Second roentgenograph in the same animal as in Figure 1 after a further and final injection of 5 cc. of thorotrast. The arteries are still conspicuous. The veins are now visualized in the normal left leg. $\times \frac{1}{4}$.
- FIG. 3. Roentgenograph taken 30 seconds subsequent to the last injection. The arteries are no longer visualized, but the veins are now clearly outlined in both legs and have commenced to fade in the normal left leg. $\times \frac{1}{4}$.





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Harman

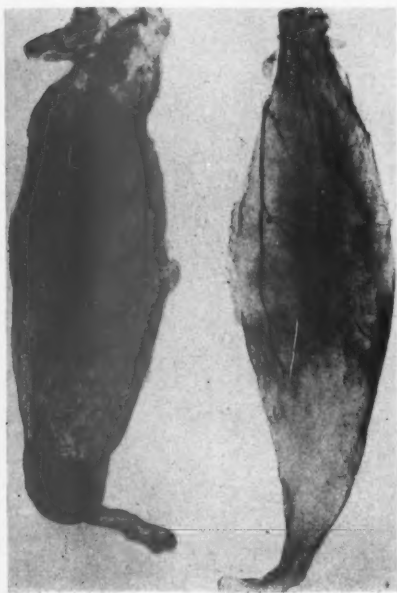
Ischemic Necrosis in Skeletal Muscle

PLATE 115

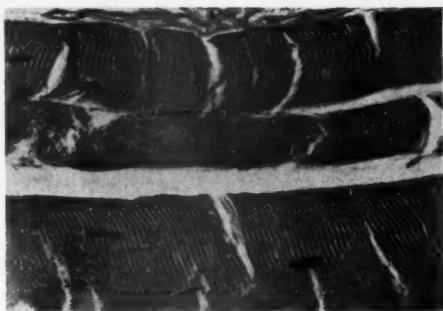
- FIG. 4. After India ink is injected into aorta, extensor muscles then are excised and cleared by Spalteholz' method. The small intramuscular arteries are clearly defined by the India ink, down to even minute ramifications in both muscles. Abnormal muscle had been ischemic for 4 hours. $\times \frac{1}{4}$.
- FIG. 5. Fibers from tibialis anticus muscle released for 24 hours subsequent to 4 hours of ischemia. Longitudinal striations are absent. The fibers are split transversely into Bowman's anisotropic disks, are thick and individualized. Nuclei are tigroid due to clearing of nucleoplasm and clumping of chromatin. Hematoxylin and eosin stain. $\times 400$.
- FIG. 6. Extensor muscles from a normal leg (on the right) and from a leg made ischemic for 3 hours with a subsequent recovery period of 21 days. Normal muscle is larger, less firm, and less deeply stained with dye. No infarct was seen in the ischemic muscle. $\times \frac{1}{2}$.
- FIG. 7. Plantares muscles from the legs of an animal which had ischemia of one leg for 8 hours. Released for 18 days before excision. The pathologic muscle consisted entirely of an infarct, except for a small area adjacent to the tendinous insertion. $\times \frac{1}{2}$.
- FIG. 8. Edge of an infarcted area of 30 days' duration. Circulation had been released after ischemia of 6 hours. The infarct is composed of fibers split into Bowman's disks. Surrounding the infarct is a compact zone of fibroblastic connective tissue. Nuclei are absent in the infarcted area. Hematoxylin and eosin stain. $\times 400$.



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Harman

Ischemic Necrosis in Skeletal Muscle

THE PERIODIC ACID ROUTINE APPLIED TO THE KIDNEY *

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(From the Department of Pathology, Medical College of Alabama, Birmingham 5, Ala.)

This communication presents results of a study of the kidney with the aid of an original staining method which has been described previously¹ for the demonstration of mucin (Fig. 1).

In brief, microscopic sections are treated with an aqueous solution of periodic acid and then colored with Schiff's reagent for aldehydes. Schiff's reagent or leuko-basic fuchsin is a straw-colored solution which is produced by the action of sulfurous acid on an aqueous solution of basic fuchsin. The reagent takes on a red or violet color when an aldehyde is added. In tissues it forms a red or violet insoluble compound at the sites where aldehyde is present. This property is utilized in various histochemical technics. In the classical Feulgen's test, the aldehyde which is formed from desoxyribose nucleic acid by hydrolysis with weak hydrochloric acid is colored with Schiff's reagent. In similar fashion, Bauer's test for the demonstration of glycogen makes use of Schiff's reagent, aldehyde being produced by the action of chromic acid.

Since periodic acid has been known to produce an aldehyde when acting upon a carbohydrate² and when acting upon serine, threonine, or hydroxylysine,³ it seemed a natural sequence to test it on tissue sections. It has been reported already that the following materials are colored by Schiff's reagent after the action of periodic acid: mucin in the intestinal and respiratory tracts, the colloid of the pituitary stalk and thyroid, mucous salivary glands, certain cells of the anterior hypophysis, and the basement membrane of the renal tubules and glomeruli.¹ The present communication describes the technic in greater detail, including the preparation of the reagents, and reports further results in the application of the periodic acid Schiff's reagent routine to the kidney.

MATERIALS AND METHODS

The sections of kidney which were studied came from young adult males, killed in the European campaign, and from the autopsies of the Jefferson-Hillman Hospital. The material had been handled in a variety of ways, although most of it had been fixed in formol-saline or in Zenker's-formol solution. A cobalt-calcium-formol fixative⁴ was found useful, particularly when postchromed by leaving for 24 to 48

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Received for publication, June 2, 1947.

hours in 3 per cent dichromate and washing in running tap water for 12 to 24 hours before dehydration. This is the method of choice when the granular cells of the renal arteriole are to be studied. The tissues were dehydrated in alcohol and embedded in paraffin after either toluene or xylene. Some tissues which had been in formaldehyde solution for a long time gave satisfactory appearances after postchroming. It may be noted here that postchroming appeared to improve the histologic and cytologic detail after nearly all fixatives.

The preparation of the necessary reagents is not a complicated matter.

The routine used to prepare Schiff's reagent is as follows:

1. Weigh out 1 gm. of basic fuchsin
2. Weigh out 1 gm. of anhydrous sodium bisulfite
3. Boil 200 cc. of distilled water
4. Add fuchsin and stir
5. Cool to 50° C.
6. Filter
7. Add 20 cc. of normal HCl (98.3 cc. of HCl (sp. gr., 1.16) made up to 1 liter)
8. Cool to 25° C.
9. Add sodium bisulfite

Keep in the dark. The fluid takes 1 or 2 days to become orange, or straw-colored, when it is ready for use.

Sulfurous acid rinse:

- 6 cc. of 10 per cent sodium metabisulfite
- 5 cc. of normal HCl
- 100 cc. of distilled water

These directions are those of Dr. John R. Baker (personal communication) and derived from Lison.⁵

The routine used for coloring the basement membrane is as follows: Paraffin sections cut at 3 to 6 μ are brought through xylene and graded alcohol to water. When the tissue has been hardened in a fixative containing mercury, the customary treatment with 0.5 per cent of I₂ in 70 per cent alcohol and 5 per cent sodium thiosulfate is carried out and the sections are washed for several minutes in running tap water. They are placed in a solution of 0.5 per cent periodic acid for 2 to 5 minutes at room temperature and then washed in distilled water briefly, although long washing in tap and distilled water does not appear to influence their appearance. They are placed for 15 minutes in Schiff's reagent at room temperature. After removal, the sections are treated for 2 minutes in each of three changes of sulfurous acid as in the classical Feulgen's test. Departure from the original technic consists of washing the sections, after the last sulfurous acid rinse, in a staining dish through

which a stream of tap water is running, for 5 to 10 minutes. This has been found to enhance considerably the brilliance of the coloration. The sections may then be brought through graded alcohols to xylene and mounted in balsam or, if a counterstain is desired, they may be placed in Harris' hematoxylin for 30 seconds and then washed thoroughly in a stream of tap water for 5 to 10 minutes before being dehydrated and mounted. If a trichrome effect is desired, the sections, after being washed following the hematoxylin nuclear stain, are placed in 0.1 per cent light green for 15 seconds or less and then washed in water before being dehydrated.

RESULTS

The basement membranes of the glomeruli and of the tubules are colored bright red or purple, as are the cell outlines of the smooth muscle cells of the arterioles and capillary walls. Ordinary connective tissue stains little if at all and there is no nuclear coloring without a counterstain. Elastica is not stained nor are the erythrocytes or the cytoplasm of cells apart from the proximal tubule. The "brush" border of the cells of the first convoluted segment is colored constantly and the cytoplasm of these cells colors to a degree which varies from one nephron to another and from case to case. There is some coloring of the cytoplasm of polymorphonuclear leukocytes and of isolated droplets or granules in various parenchymal cells, frequently in the position of the Golgi element.

Counterstaining colors the chromatin blue to black. Light green stains the cytoplasm of the cells and the erythrocytes, and also the basement membrane to some extent, particularly in the glomerulus. The granules of the afibrillar arteriolar cells in the "crush" kidney are colored a bright red, somewhat lighter in shade than that of the basement membranes. When it is desired to study the arteriolar granules, it is necessary to obtain tissue within 1 hour after death, and the cobalt-calcium-formol fixative seems the best, with postchroming essential. Alcohol-toluene or alcohol-xylene methods of dehydration and clearing must be used.

The normal glomerulus shows a single basement membrane by this technic (Fig. 2). It is believed that glomerular structure can be interpreted more thoroughly with the present technic than in sections prepared by the trichrome stain or by modifications of Mallory's aniline blue-orange G mixture.

In the "crush" kidney there is a strong prominence of the granular cells of the renal arterioles, as Goormaghtigh⁶ has described. The

fibrin of venous thrombi (Fig. 3) stains strongly by this method. The hyalin-appearing casts in the tubules stain with varying degrees of brilliance. Glycogen and amyloid are colored brightly.

The alteration of the basement membrane of the glomerulus in arteriosclerosis, which was described by McGregor,⁷ is made prominent (Fig. 4).

The hyalin of arteriolosclerosis (Fig. 5) shows strong coloring. Hyaline droplets (Fig. 6) in epithelial cells of the proximal tubules color brilliantly. Where tubules have become atrophied in scars, there is persistence of staining of the basement membranes of the tubules except in the oldest scars. It was concluded from the study of many cases that thickening of the basement membrane of the corresponding tubule is an early change which occurs in any progressive glomerular injury.

DISCUSSION

Three features appear to be worthy of discussion at the present time.

The Utility of the Method as a Histologic Aid in the Study of the Kidney

The basement membranes of glomeruli and tubules are so well shown by this technic that it is proposed as the method of choice. Fresh tissues, from autopsies performed within 1 hour after death, are best. When autopsy is delayed, the postchroming in 3 per cent potassium dichromate for 24 hours followed by washing of the tissue in running water for the equivalent time produces satisfactory results.

A particular advantage of the method is that no "differentiation" of the section is necessary. This is in contrast to the aniline blue-orange G and Masson's trichrome technics in which the end result is a personal technical production. With them, differences in results may be produced by minor variations in the length of differentiation or of staining and by minor variations in the quality of the dye. Serial sections cut at quite long intervals of time give identical results with the periodic acid routine.

The Structure of the Normal Glomerular Basement Membrane

The glomerulus in the normal kidney shows a single basement membrane by the present technic. The glomerular alterations in a variety of diseases which have been studied appear to be capable of explanation upon this basis. Nothing is revealed with any certainty as to the nature of the basement membrane of the glomerulus or of the tubules. It is of interest in connection with the pathogenesis of Bright's disease

and other affections of the kidney that a coloring similar to that seen in the basement membrane is observed in the hyalin of arteriosclerosis, in casts, and in hyaline droplets.

The Histochemical Validity of the Present Method

When the periodic acid technic was described originally, it was pointed out that it was of histologic rather than of histochemical usefulness. That is still the present position. Malaprade² had introduced periodic acid into quantitative chemistry, having found that it would produce an aldehyde when it acted upon the connection between two carbon atoms of a chain if each of these two adjoining carbon atoms had a hydroxyl group. Nicolet and Shinn³ found that the bond between adjoining carbon atoms was broken and that an aldehyde was formed when one carbon atom had a hydroxyl group and the other an amino group. The demonstration of aldehyde in tissue sections by Schiff's reagent after the action of periodic acid was a logical attempt, but histochemical conclusions are difficult to reach because of the following two facts. Lison⁵ has shown that a great variety of materials other than aldehydes, notably ketones and unsaturated compounds, will recolor Schiff's reagent. Secondly, many substances in tissues contain one or both of the linkages from which periodic acid can produce aldehyde. Separate identification of glycogen, glycoprotein, glycolipid, and the three amino acids (serine, threonine, hydroxylysine) is necessary if the method described is to give valid histochemical data. It is my opinion that the basement membrane consists of a carbohydrate-protein compound of the mucoprotein type, but decision must be deferred for the reasons given.

SUMMARY AND CONCLUSIONS

In sections of normal human kidneys the basement membranes, capillary walls, and the outlines of the smooth muscle cells of the arterioles are colored with Schiff's reagent after periodic acid.

Besides these structures, the same routine applied to abnormal kidneys colors the following materials: The hyalin of arteriosclerosis, hyaline casts, glycogen, amyloid, colloid droplets in tubular epithelium, and the granules of the afibrillar cells of the arterioles.

The material of the basement membrane which takes the stain is believed to be a mucoprotein. Opposed to immediate acceptance of this hypothesis are (1) the nonspecificity of the recoloring of Schiff's reagent, and (2) the production of aldehyde by periodic acid from three amino acids.

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DESCRIPTION OF PLATES

All illustrations were made from sections treated with periodic acid followed by Schiff's reagent.

PLATE 116

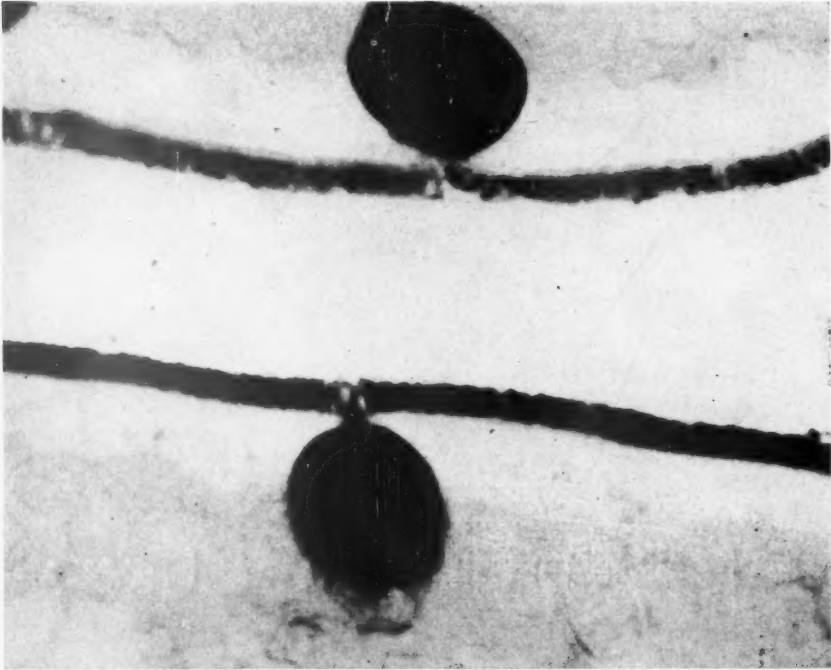
- FIG. 1. Human jejunum. There is coloring only of mucous goblets and of the free surface ("brush border") of the intestinal epithelium. $\times 1500$.
- FIG. 2. Normal glomerulus and arteriole from a white female, 42 years of age. The basement membrane of the glomeruli and tubules is colored as well as the cell outlines in the arteriole. There is slight coloring of the free surface of the proximal convoluted tubules. $\times 520$.

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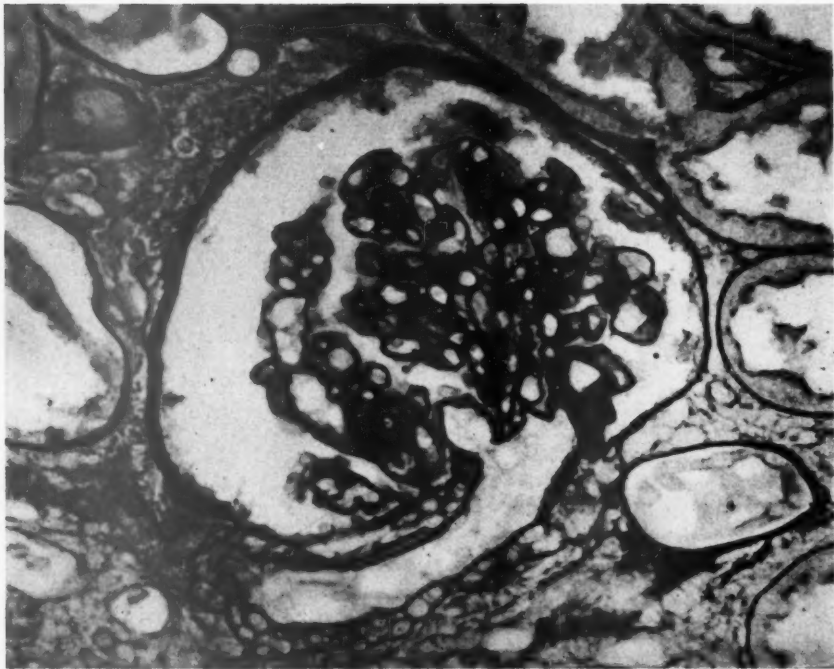
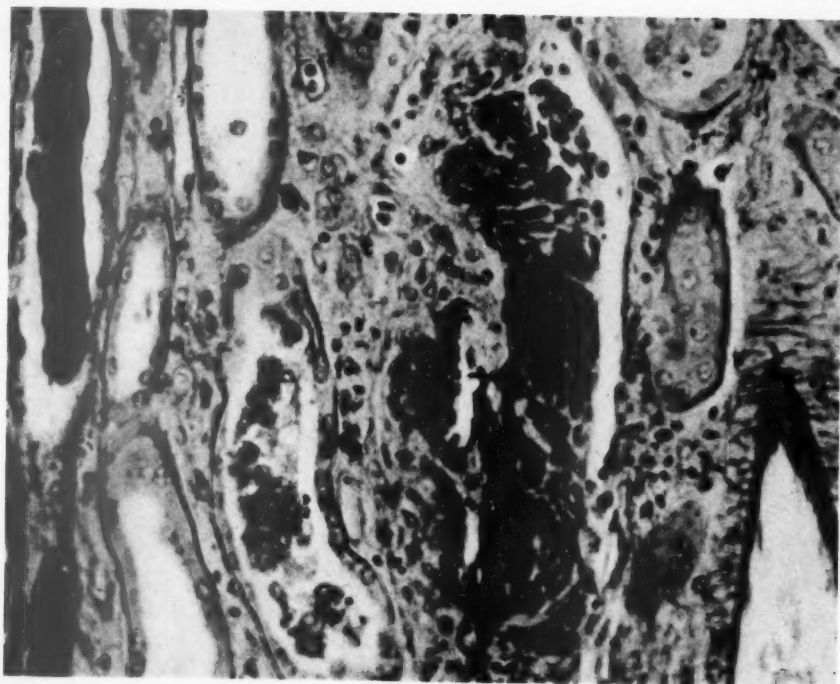


PLATE 117

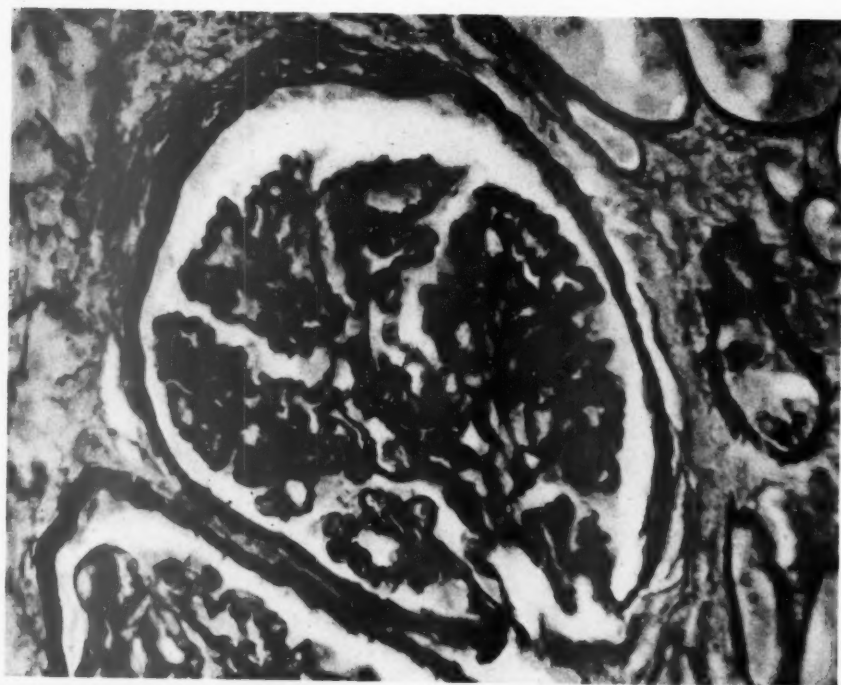
FIG. 3. Tubular injury and venous thrombosis after a severe wound. A thrombus can be seen protruding into the venule from the inflamed interstitial tissue. There is a hyaline cast in one tubule and some cellular debris in another. Counterstained with hematoxylin. $\times 320$.

FIG. 4. Glomerulus from a case of essential hypertension in a colored female, 36 years old. Death in second cerebral hemorrhage. Most of the glomeruli, like the one of which a corner is shown, appeared normal; a few—one-fourth to one-fifth—showed the wrinkling of the simplified basement membrane which is illustrated. Hyaline arterioles (not shown) were numerous. $\times 520$.

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McManus

Periodic Acid Routine Applied to Kidney

PLATE 118

FIG. 5. Area from the generally normal kidney of a white female, 46 years old, dying with metabolic craniopathy. An obsolescent glomerulus is seen. There are several segments of a tubule in process of disappearance and showing thickening of the basement membranes. The arteriole shows several patches of hyalin. $\times 260$.

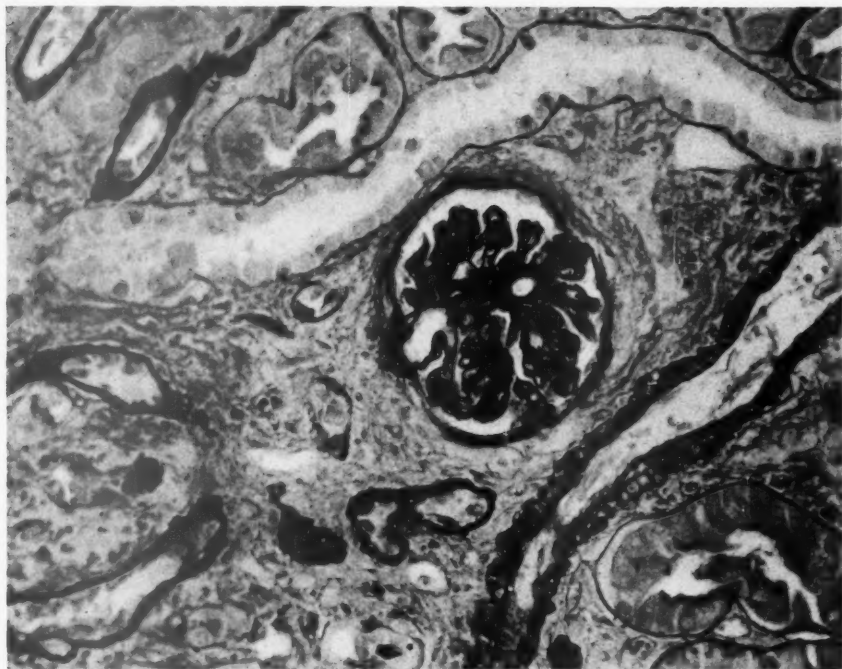
FIG. 6. From the same case as used for Figure 5. Several tubules, the cells of which contain hyaline droplets, are seen. One other tubule is in process of disappearance. It shows a greatly thickened basement membrane and a diminished or absent lumen. $\times 950$.



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McManus

Periodic Acid Routine Applied to Kidney

EPITHELIUM-LIKE INCLUSIONS IN THE HEART *

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The pathologic anatomist subscribes to the view, common to all sciences, that the exceptional condition is worthy of study, since it frequently is of aid in understanding the more usual deviations from normality. The report, then, of a clinically insignificant, but anatomically interesting, cardiac anomaly justifies itself. As so often happens and as remarked by Davidsohn,¹ the lesion was accidentally discovered at necropsy, and only microscopically.

REPORT OF CASE

Clinical History

A white married woman, 29 years of age (no. 46-7801), entered the St. Joseph Hospital, October 21, 1946. She had difficulty in breathing, generalized pain in the chest and upper part of the abdomen, cough, and weakness. These symptoms had recurred frequently since their onset in 1940. When she was 14 years old (1931) she showed evidence of heart disease; there was no definite history of rheumatic fever. In the last 6 years of life she bore three living normal children, the last having been born 6 weeks before death.

During the 3 days in which she was hospitalized before her death, the temperature was always subnormal, and the respirations moderately rapid. Except for a brief transient rise, the pulse rate was never above 66, and terminally was recorded as 60. Treatment was ineffective. The symptoms became rapidly worse, and the patient expired on October 24, 1946.

Necropsy Findings

The body was examined (no. 46-A-083) 3½ hours after death. There was universal acute passive congestion, and fluid was found in the pleural and pericardial cavities, and in the abdomen. The enlarged heart (515 gm.) was dilated. Rheumatic stigmata included mitral valvular scarring with insufficiency, and bilateral atrial mural endocarditis and pulmonary arteritis.

In the original microscopic section of the posterior leaflet of the mitral valve and adjacent left atrium and ventricle an unusual feature was noted with the naked eye. In the angle between atrium and ventricle (Fig. 1), in the general plane of the valvular ring, there were round, solid and hollow, smaller and larger areas stained blue by hematoxylin. Closer examination of this, as well as of additional sections from the same block, failed to uncover any physical bridge between these structures and either the endocardium or epicardium. The inclu-

* Received for publication, June 30, 1947.

sions extended irregularly for a very short distance into the atrial myocardium, but did not do so on the ventricular side. All were sharply circumscribed by the connective tissues in which they lay.

The basic cell of the anomaly had a varied shape. In the solid structures it was generally polyhedral, with a cuboidal form assumed by those in the peripheral layer or layers. The hollow formations usually were lined by cuboidal cells; the lumina could be assumed to have been formed by dissolution of the cells in the interior.

Centrally placed in each cell was a round to slightly oval, single nucleus with distinct limiting membrane. The chromatin was finely granular and well dispersed. At least one obviously larger chromatin granule was found in every nucleus, and a few nuclei had single, well formed nucleoli. Nucleolar formation was accompanied by thickening of the nuclear membrane, and by coarsening and concentration of the chromatin on the membrane. A few mitotic figures were identified.

The cytoplasm was moderately eosinophilic, dense, and nongranular. No cytoplasmic inclusions were seen.

Hollowing of the cellular formations was accomplished by disintegration of the central portions. Some cells exhibited pyknosis and cytoplasmic hyalinization. Others, greatly swollen, with multiple vacuoles, usually exhibited a distinct, thick, cellular membrane before disintegration. A few cells crumbled without going through any of these processes.

The larger spaces (Fig. 2), many of them confluent, were well filled with cellular detritus and cells in varied stages of degeneration. A few neutrophilic and eosinophilic leukocytes and an isolated red cell completed the picture. In a few lumina conglomerated eosinophilic material was partly or completely calcified.

Adjacent to the ventricular epicardium (Fig. 3) the early stage of luminal formation lent the appearance of sebaceous glands (Fig. 3). The central cells were greatly enlarged and the cytoplasm vacuolated almost to the point of disappearance. The nuclear membranes stood out as thick eosinophilic rings, and the centrally placed single nuclei were slightly shrunken. No intercellular bridges were identified.

Another space was filled peripherally with several concentric rings of acellular hyaline material about a central mass of cloudy amphoteric substance, probably lightly impregnated with lime salts.

The stroma of the area was dense, generally collagenous, poorly cellular, and very vascular. The numerous vessels were chiefly capillaries and arterioles, but a few small arteries were seen. Lymphoid

cells and fewer plasma cells were congregated in appreciable numbers in the neighborhood of the large hollow formations.

DISCUSSION

The recent paper of Anderson and Dmytryk² thoroughly reviewed most of the available literature on primary cardiac tumors, as well as that dealing with epithelium-like inclusions, some similar to those described in this paper. In their case of a myxomatous tumor (the result of changes in thrombotic material) of the right atrium, gland-like and cystic structures were found in the new growth. These structures were lined by a variety of cells, ranging from flat cells of endothelial character to those of columnar type. Mucicarmine-positive material was identified in the latter, and cilia were suggested but not positively recognized. The inclusion of pericardial elements in the atrial wall during cardiac development was suggested as the causative factor.

Rezek³ was kind enough to furnish slides of the case he reported in 1938, and also to review material of the lesion here recorded. In his sections the epithelium-like character was, in general, not as pronounced as in the illustrations in his paper. He called attention⁴ to the sebaceous gland-like character of some structures in the present case, as described above, and inquired whether these were "predestined to develop into skin." Notwithstanding superficial appearances, we are reluctant to accept this suggestion, particularly in the absence of intercellular bridges.

The cellular spaces illustrated in the publication of Perry and Rogers⁵ closely resemble those of the case reported here. This was confirmed by Rezek,³ who had received slides from these authors after they had published their findings. He noted solid nests with cells like those of sebaceous glands or vacuolar degenerated epithelial cells. Perry and Rogers' diagnosis was "lymphangio-endothelioma," and histogenesis was ascribed to "lymphatic vasoformation." Rezek, in the text of his own original report, spoke of lymphangio-endothelioma. He added, however, that if the structures were epithelial, they might have resulted from the fetal inclusion of adjacent tissues, like the foregut (esophagus). The title of his paper showed the weight of his opinion by the inclusion of the phrase, "primary epithelial tumor."

Cornifying stratified squamous epithelium and sweat-gland-like apparatus were found by de Châtel⁶ who referred to ectodermal heterotopia, or metaplasia of retained entodermal cells. Kolatschow,⁷ David-

sohn,¹ and Bayer⁸ each described cysts lined by ciliated columnar epithelium in the left ventricular papillary muscle. Kolatschow spoke of dystopia, as well as the possibility of an epicardial anlage with metaplasia to cylindrical epithelium. Davidsohn did not support any theory, and Bayer suggested a common origin with the bronchial wall.

We have no theories of our own. The propinquity, early in fetal life, of epithelium-producing tissues to the cardiac structures renders dystopia, heteropia, or inclusion³ especially attractive. In the absence of definite identification of the abnormal formations as epithelial, an endocardial, or epicardial origin seems more reasonable. The paucity of published observations on such solid and hollow inclusions makes the formulation of opinion additionally difficult. One suspects the meagerness is real, rather than relative, because the heart is more intensively and extensively studied at necropsy than any other organ.

SUMMARY

In the subepicardium and adjacent left atrial myocardium in the plane of the mitral ring of a white woman with rheumatic heart disease, abnormal structures were found. These were both solid and hollow, and had an epithelial character which could not be established beyond doubt. A cardiac origin, including epicardium and pericardium as possible sources, is probable, in consonance with similar, previously published reports.

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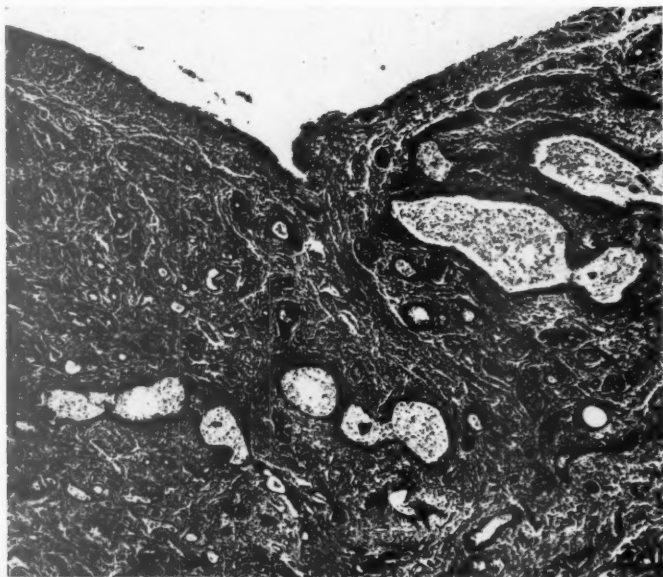
[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 119

- FIG. 1. Epicardium and subepicardium of the left atrioventricular angle, showing solid and cystic epithelium-like elements. Hematoxylin and eosin stain. $\times 32$.
- FIG. 2. Portion of the subepicardium showing cystic epithelium-like structures. Desquamated and broken-down lining cells form the contents of the cyst; calcification of intracystic necrotic material. Hematoxylin and eosin stain. $\times 50$.
- FIG. 3. Epicardial layer and subepicardial structure suggestive of sebaceous gland. Hematoxylin and eosin stain. $\times 215$.

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FORTY-FIFTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

PHILADELPHIA

MARCH TWELFTH AND THIRTEENTH, 1948

THE AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

Forty-Fifth Annual Meeting,
Jefferson Medical College,
Philadelphia, Pennsylvania

March Twelfth and Thirteenth, 1948

PRESIDENT SOULE IN THE CHAIR

BUSINESS MEETING

March Twelfth, 1948

For the Council, the Secretary announced the following actions:

Election of new members

| | |
|--|--|
| Carlton Auger, Quebec City, Que. | Thomas J. Moran, Danville, Va. |
| William H. Bauer, St. Louis | Gert L. Laqueur, San Francisco |
| Joseph L. Bernier, Washington, D.C. | Pablo Mori-Chavez, Lima, Peru |
| Maurice M. Black, Brooklyn | Joseph I. Mossberger, Denver |
| Charles Breedis, Philadelphia | Hans N. Naumann, Taunton, Mass. |
| Charles W. Buggs, Detroit | Manuel D. Peñas, St. Louis |
| Francis C. Coleman, Des Moines | Louis C. Posey, Birmingham, Ala. |
| John T. Cuttino, Durham | Dexter L. Reimann, Baltimore |
| Richard Ford, Boston | Russell A. Runnells, East Lansing, Mich. |
| Ira Gore, Washington, D.C. | Olaf K. Skinsnes, New York |
| John D. Hamilton, Kingston, Ont. | Reuben Straus, Beverly Hills, Calif. |
| Philip H. Hartz, Willemstad, Curaçao, N.W.I. | W. T. S. Thorp, Bethesda, Md. |
| Eugene Hildebrand, Great Falls, Mont. | J. P. Tollman, Omaha, Neb. |
| Arthur A. Humphrey, Battle Creek, Mich. | Richard C. Wadsworth, Bangor, Me. |
| Oscar B. Hunter, Jr., Washington, D.C. | Prem N. Wahi, Agra, U.P., India |
| Aaron Kellner, New York | Frederic J. Wohlwill, Hathorne, Mass. |
| Benjamin H. Landing, Boston | Charles L. Yuile, Rochester, N.Y. |
| | Edwin E. Ziegler, Lancaster, Pa. |

Reinstatement to membership of Drs. Harold M. Dixon and John W. Miller.

Acceptance, with regret, of the resignations of Drs. Warren C. Corwin, Gilbert Dalldorf, Harold W. Lyall, Peter K. Olitsky, and Ralph G. Stillman.

With deep regret, the deaths of Drs. Clement C. Fenton, Robert Green, Robert N. Nye, Cornelius A. Hospers, Francis P. Parker, and O. T. Schultz.

Upon nomination of the Council, the Association elected the following officers:

| | |
|-----------------------------------|--------------------|
| <i>President</i> | E. W. GOODPASTURE |
| <i>Vice-President</i> | SHIELDS WARREN |
| <i>Secretary</i> | HOWARD T. KARSNER |
| <i>Treasurer</i> | ALAN R. MORITZ |
| <i>Assistant Secretary</i> | HERBERT Z. LUND |
| <i>Assistant Treasurer</i> | SIDNEY FARBER |
| <i>Incoming Member of Council</i> | WILLIAM H. FELDMAN |

The President announced that the American Type Culture Collection is an actively growing solvent concern housed in its own building in Washington. He said that at present there are not less than 5,000 cultures. Duplicate cultures are kept in a separate repository so that if those in the building are destroyed it will be possible to replace them. It was pointed out that the entire income is from sale of cultures. Dr. Soule suggested that members who receive requests for cultures refer these requests to the American Type Culture Collection, 2029 M Street, N.W., Washington, D.C., so far as possible.

The Secretary announced the re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the ensuing year, and the election of Dr. Tracy B. Mallory to the Editorial Board for a period of six years.

The Secretary announced the nomination of Dr. Malcolm H. Soule as representative of The American Association of Pathologists and Bacteriologists in the Division of Medical Sciences, National Research Council, for a term of three years beginning July 1, 1948.

For the Council, the Secretary announced that the next meeting of the Association will be held in Boston on April 15 and 16, 1949. The topic for the symposium for the 1949 meeting is "Pathology in Forensic Medicine." Dr. Alan R. Moritz has been appointed to act as referee.

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted by the Council. It was accompanied by a certification from the Edwin S. Morse Company, Public Accountants, Boston. In condensed form, the Treasurer's report follows:

General Checking Account
Receipts

| | | |
|---|-------------|-------------|
| Balance on hand, January 1, 1947 | | \$ 2,740.64 |
| Membership dues: | | |
| Current | \$ 7,290.00 | |
| Back | 90.00 | |
| Advance (1948) | 30.00 | |
| Interest on bonds, from investment account | 590.00 | |
| Sale of U.S. bonds, from investment account | 6,044.04 | |
| | | 14,044.04 |
| | | \$16,784.68 |

Disbursements

| | | |
|---|-----------|-------------|
| American Journal of Pathology (\$8.00 per member) | | \$ 5,456.00 |
| C. E. Lennon (Secretary to Dr. Karsner) | \$ 200.00 | |
| P. A. Glass (Secretary to Dr. Moritz) | 150.00 | |
| Reporting 1947 meeting | 213.08 | |
| Attending meetings (officers) | 192.32 | |
| General office expense (secretary), including expense for annual meeting | 212.37 | |
| General office expense (treasurer) | 44.30 | |
| National Society Medical Research | 50.00 | |
| Auditing service | 30.00 | |
| Safe deposit box | 6.00 | |
| Bank charges | 3.44 | |
| Transfers to investment account | 8,044.04 | |
| | | 9,145.55 |
| | | \$14,601.55 |

Balance on hand, December 31, 1947 \$ 2,183.13

Investment Account

| | | |
|-----------------------------------|-----------------|-------------|
| Balance, January 1, 1947 | | \$32,155.12 |
| | <i>Receipts</i> | |
| Interest, savings bank accounts | \$ 114.08 | |
| Gain on U.S. bonds sold | 44.04 | |
| Franklin Savings Bank | 4,000.00 | |
| Provident Institution for Savings | 4,000.00 | |
| | | 8,158.12 |
| | | \$40,313.24 |

Disbursements

| | | |
|---------------------------------|-------------|-------------|
| U.S. bonds sold (3% of 6/15/48) | \$ 6,000.00 | 6,000.00 |
| Balance, December 31, 1947 | | \$34,313.24 |

Inventory of Investments
December 31, 1947

| | | |
|-----------------------------------|-------------|-------------|
| U.S. bonds 2½, series G | \$20,000.00 | |
| Provident Institution for Savings | 4,000.00 | |
| Franklin Savings Bank | 4,000.00 | |
| Cambridge Savings Bank | 4,216.17 | |
| National Shawmut Bank | 2,097.07 | |
| Total | | \$34,313.24 |

SCIENTIFIC PROCEEDINGS

A STUDY OF THE BEHAVIOR IN TISSUE CULTURE OF LYMPH NODES FROM HODGKIN'S DISEASE. Antonio Rottino, and (by invitation) Barney Worken and Augusta Hollender, New York, N.Y.

Abstract. Lymph nodes from 25 patients with Hodgkin's disease and 26 patients with non-Hodgkin's disease were studied in tissue culture.

In the Hodgkin's series, the following were conspicuous: liquefaction of medium, formation of multinuclear giant cells, phagocytosis, and occurrence of intracytoplasmic inclusions.

For the ensuing discussion we shall enlarge on the phenomenon of liquefaction. The conclusions drawn from this study are:

1. Liquefaction of medium in tissue culture is nonspecific.
2. It is more frequent and of greater degree in Hodgkin's than in non-Hodgkin's cultures.
3. Liquefaction occurs more often and to a greater degree with the granulomatous form of Hodgkin's disease and is diminished as the node becomes more fibrotic and sarcomatous.
4. Liquefaction probably represents digestive activity on the part of a fibrinolytic enzyme liberated from cells of one or more types. In Dr. Worken's opinion the ferment is elaborated by the reticular cell.

Discussion

(Dr. Herman Hoster, Columbus, Ohio) As I interpret Dr. Rottino's remarks concerning liquefaction, they appear to nullify, at least in part, the observations of Grand based on liquefaction. I should like to ask whether the inclusion bodies described by Grand are also considered nonspecific. I should like to inquire also whether there are any changes which Dr. Rottino considers specific in these tissue cultures.

(Dr. Jacob M. Ravid, New York, N.Y.) I should like to ask Dr. Rottino whether he has done any multiple tissue cultures on patients who have had several biopsies at various times during their illness, and if so, whether he observed in such cases any differences with regard to the liquefaction phenomenon.

(Dr. Rottino) With regard to the inclusion bodies, all sorts of inclusion bodies are seen in tissue cultures of lymph nodes from Hodgkin's disease. The same, however, are seen in control material. One source for cytoplasmic inclusions is the disintegrated remnants of cells dying in tissue culture and their subsequent phagocytosis by multinucleated giant cells and mononuclear cells. Present histochemical methods are not sufficiently refined to differentiate the histogenesis of all the inclusions that are found within cytoplasm. At present I think that most of the inclusions which we see arise by phagocytosis.

With regard to any specific phenomena occurring in Hodgkin's disease, I confess we have not seen any. The occurrence of Sternberg-Reed cells is mentioned; as far as I can make out, however, what have been identified as Sternberg-Reed cells are in my opinion multinucleated giant cells of the foreign body type that make their appearance in response to debris that occurs in tissue cultures.

In reply to Dr. Ravid's question concerning multiple biopsies and multiple cultures, we have observed cultures of different nodes removed at different times.

In 2 individuals the second culture showed the formation of numerous large multinucleated giant cells not present in the first cultures. In another instance in the first culture liquefaction was much more common than in the second culture.

HISTOPATHOLOGIC OBSERVATIONS IN CASES OF HODGKIN'S DISEASE TREATED WITH NITROGEN MUSTARD.* Virgil H. Cornell and (by invitation) A. S. Blauw, Washington, D.C.

Abstract. Several articles have reported temporary remissions, and a few relatively long periods of remission, in cases of Hodgkin's disease treated with nitrogen mustard. These have given no detailed descriptions of histopathologic changes subsequent to treatment, nor commented upon the absence of any changes. Other publications of animal experimental work during the war years, which have been recently released for publication, describe changes in great detail.

Seventeen cases, in which material before and after nitrogen mustard therapy was available, have been studied and no consistent histopathologic change can be found which may be attributed to the therapy. The present report is made to record the negative findings and warn against application of the animal tissue changes after sublethal dosage to the human cases treated by therapeutic dosage.

Discussion

(Dr. Antonio Rottino, New York, N.Y.) I had the privilege of doing an autopsy on a patient with Hodgkin's disease who died 3 days after a course of nitrogen mustard. The case was a late one. There were complications, such as a lobar pneumonia and severe anemia and leukopenia. At autopsy there was necrosis not only of the lymphocytes but also of the reticulum cells of all the lymph nodes we could find, as well as in the bone marrow.

UNUSUAL CHANGES IN LYMPHOSARCOMA UNDER NITROGEN MUSTARD THERAPY. Oscar B. Hunter, Jr. (by invitation), Washington, D.C.

Abstract. This paper deals with an apparent coagulation necrosis of lymphosarcoma tissue in a number of cases seen at the Army Institute of Pathology. These cases all show the same necrotizing effect of the tumor tissue without apparent effect on the relatively normal tissue. This effect has not been noted in the literature to date and in the instances detailed in the paper the tumor has apparently been eradicated almost completely.

Discussion

(Dr. V. H. Cornell, Washington, D.C.) I would like to say that the apparent discrepancy in the two papers which have just been presented may be reconciled. We have seen this change in autopsied cases given nitrogen mustard therapy; however, I think I have seen it also in cases that have never met nitrogen mustard. It was with this idea in mind that for over a year and a half I have been going back over our material and every time I saw something in cases that were treated with nitrogen mustard, I saw it also in cases that were not treated. I think that unless you look at this new treatment with great discrimination you cannot be sure that the criteria you are applying are specifically related to the therapy. I think there must be some action, and apparently some late action, of nitrogen mustard. It is very well described in animals with large

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

doses. As I stated, the effect ceases, perhaps, in 15 to 30 minutes within the body. Clinically the improvement is prompt but anatomically delayed. What I was afraid of was that the papers, which have been in preparation during the last year and are now coming out in the literature, on animal therapy with huge doses of nitrogen mustard might be interpreted as indicating what was occurring in the patient who receives the therapeutic dose. I simply urge everyone to be extremely strict in the interpretation of what they see.

(Dr. Shields Warren, Boston, Mass.) May I ask Dr. Cornell if he means to imply that the type of phagocytosis he showed is specific for the action of nitrogen mustard?

(Dr. Cornell) No, I thought I made that clear; the first group of slides showed phagocytosis and were nitrogen mustard cases, while the last three slides were from cases that had not had nitrogen mustard. I am simply trying to show the similarity of phenomena that were observed first in the nitrogen mustard cases, and could then be found in other cases.

(Dr. Hunter) I am glad we had a second biopsy in the first case, showing the histologic change before and after. It was very closely controlled.

ATYPICAL AMYLOID DISEASE, WITH OBSERVATIONS ON A NEW SILVER STAIN FOR AMYLOID.* Lester S. King, Chicago, Ill.

Abstract. From the group of so-called "primary" amyloidosis, a special subgroup of "atypical amyloidosis associated with senility" is distinguished. Five cases, all concerning patients over 80 years of age, are described, in which amyloid occurred in the heart, almost exclusively, and which showed no common denominator apart from advanced age. These are contrasted with a further case of "atypical amyloidosis associated with pyelonephritis." A new method of staining amyloid with ammoniacal silver is described.

Discussion

(Dr. Alfred Plaut, New York, N.Y.) How were the amyloid reactions with the customary methods? I ask the question because of the well known staining irregularities of atypical amyloid.

(Dr. Jesse E. Edwards, Rochester, Minn.) I should like to subscribe to the skepticism concerning the infrequency of amyloidosis of the heart in the comments of Dr. King. I think the frequency of this condition has not been appreciated in the past. In the last year we have seen 4 instances of the condition essentially similar to the 5 he showed. In each of these 4 the diagnosis was made on gross examination and the gross diagnosis was made on the basis of lesions seen in the endocardium, especially of the left atrium. The lesions were also seen in the right atrium. They appeared as "tapioca" lesions. The little specks may be numerous; they were innumerable in the endocardium of the left atrium and were smaller than those which we ordinarily associate with the tapioca lesions of amyloidosis of the spleen; they tended to be of pinpoint size, rarely a millimeter in diameter. Occasionally some of these lesions would involve the valvular endocardium. I wonder if Dr. King found, in his cases, lesions involving the endocardium. In the 4 cases I mentioned there were also lesions of amyloid in the myocardium.

Just a word about terminology: I think the term he recommends is a little vague. I do not know that I can suggest a better one, but I would suggest that in some way the terminology should definitely include amyloidosis with reference to the heart which the term "atypical amyloid disease" does not do.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

(Dr. Ralph D. Lillie, Bethesda, Md.) May I ask Dr. King if his silver reaction is based on formaldehyde-fixed material, or are other fixatives used?

(Dr. Friederich Wohlwill, Danvers, Mass.) I would like to ask whether, by chance, the nerves have been examined. I saw a case of atypical amyloidosis with the clinical picture of polyneuritis in which the main seat of the amyloid substance was in the spinal roots, the spinal root ganglia, and the peripheral nerves. But there was amyloidosis of the heart, too.

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether Dr. King will comment on the relationship of this substance to ordinary hyalin, realizing that hyalin is a rather all-inclusive term and that amyloid itself includes quite a complicated heterogeneous group of chemical substances. I ask the question because it is my impression that this is a rather common phenomenon in hearts as I see them. Your statistics do not represent the ordinary experience of the average pathologist, at least, judging from my experience. My interpretation of the lesion is that it represents a form of change in arteriosclerosis. The lesions in the glomeruli are very similar to what we see in the Kimmelstiel-Wilson lesion of the glomeruli and in hyalinosis of arterioles, and there we are fairly well agreed that each is a variety of arteriosclerosis. The noted localization in the vessels seems to bear out that impression. I would be appreciative if Dr. King would resolve this difficulty.

(Dr. Arthur C. Allen, New York, N.Y.) In regard to Dr. Angrist's comment, I should like to re-emphasize that lesions in diabetic glomerulosclerosis which have a similarity in routine sections to the glomeruli of amyloid disease do indeed have a striking argyrophilia, but that the argyrophilic material has a specific laminated pattern, which I believe is different from the homogeneous smudgy appearance of the argyrophilia of the amyloidotic glomeruli described here.

(Dr. King) In reply to Dr. Plaut's question, I should have emphasized that in all these cases the amyloid stains very well with methyl violet and Congo red, so that the staining reactions are entirely typical.

Before replying to Dr. Edwards, may I ask him the age of the patients in whom he found the disease?

(Dr. Edwards) I do not recall the exact ages, but I believe they were all older individuals.

(Dr. King) In the matter of terminology, the best I could think of was atypical amyloidosis associated with senility, and I used that because I do not believe the amyloid is necessarily restricted to the heart. There are observations in the literature describing amyloid in the seminal vesicles. Unfortunately in these cases the seminal vesicles were not examined histologically, but in a series of reported cases in which the ages were over 80, almost 70 per cent showed amyloid in the seminal vesicles. My feeling is that amyloid deposition in the heart and in the seminal vesicles is a function of age. I have observed it in the heart. I may say that we had not known of any in the peripheral nerves.

In reply to Dr. Lillie's question, the material is formalin-fixed. The only striking feature of this impregnation is that no reduction is necessary.

In regard to Dr. Angrist's question, he is asking me a question which the pathology students are asking, and usually we find that the questions which the pathology students ask are the most difficult to answer. The only way I can tell the difference between amyloid and other hyaline substances is by specific stains. My feeling is that any hyaline material which reacts positively with the accepted amyloid stains—Congo red, methyl violet, iodine, and, I hope, this technic—is amyloid, and if it does not react with these methods it should not

be called amyloid. I agree with Dr. Angrist that this is probably a common condition. However, the importance of applying specific amyloid stains to this condition has not been appreciated. In my experience we have had 20 cases over the age of 80, and in these 4 showed this condition. I have not tried the technic on any examples of intracapillary glomerulosclerosis.

ENHANCEMENT OF METASTASIS OF A MOUSE MAMMARY CARCINOMA FOLLOWING ROENTGEN IRRADIATION. Edwin D. Murphy and Henry S. Kaplan (by invitation), Memphis, Tenn.

Abstract. The tumor studied was Dr. Halsey Bagg's tumor no. 755 which grows slowly in C57 black mice, seldom metastasizing. In 4 experiments, groups of 40 to 50 C57 mice were inoculated, and when the tumor reached a diameter of 1 cm., half of the mice received doses of 400 to 1000 r. applied locally to the tumor, the rest of the mice being held as controls. The incidence of pulmonary metastasis after 6 weeks was 44 per cent in the case of the irradiated tumors and 10 per cent in the controls. Heterotransplantation studies carried out collaterally indicated that progressive growth was more readily obtained with irradiated tumor than with untreated tumor, suggesting a primary effect upon the tumor rather than directly upon the host.

Discussion

(Dr. Alfred Plaut, New York, N.Y.) Was this the original strain from the Jackson Memorial Laboratory, or a sub-strain?

(Dr. Murphy) The strain was Strong's sub-line of the C57 black, but, as I indicated, we carried the tumor in that strain for a period of 2 years before this experiment was started. We have carried on some observations on metastasis for another type of work, and are using that as our background for the behavior of the tumor. The incidence of spontaneous mammary carcinoma is low and none appeared in any of our animals in the age group used.

COLOR AND PRECIPITATION REACTIONS WITH MALIGNANT TUMORS. Emil Weiss, Chicago, Ill.

Abstract. Fresh unfixed tissue of malignant tumors (0.25 to 0.5 cc. suffices) is cut to small particles, placed in a tube, covered with 10 cc. of a saturated solution of litmus in 70 per cent acetone, corked and shaken vigorously for 1 minute. Malignant tissue turns the solution red, while the control containing normal or benign tissue remains violet. A solution of 20 per cent salicylic acid in acetone, used in the same way, gives precipitation with malignant tumors and none with benign or normal tissues. Controls for each reaction are: (1) Reagent control containing the reagent used in the test; (2) positive control containing the reagent and a known malignant tissue; (3) negative control containing the reagent and a known normal tissue. The controls are handled in the same way as the unknown. The test is interpreted as strongly positive if salicylic acid and litmus give the described reactions for malignant tumors, as weakly positive if only salicylic acid gives the typical reaction, and as negative if precipitation does not occur with salicylic acid regardless of the litmus reaction. Impurities of proteins in the test tubes may cause false positive reactions and acids may cause false negative reactions. The test gave results corresponding with histologic examinations in 95.17 per cent of malignant tumors (80.12 per cent strongly positive, 15.06 per cent weakly positive reactions). Benign tumors gave

4.24 per cent positive reactions (only weakly positive). The test applies to all types of malignant tumors.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. Weiss if he has tried this reaction on serum, or urine, or on extracts of normal tissue.

(Dr. Weiss) This reaction cannot be used on serum because it would coagulate the serum. For urine tests the reagents would have to be used in a more concentrated form and adjusted accordingly. Extracts of 200 normal tissues gave satisfactory results.

GRANULAR CELL "MYOBLASTOMAS" AND GRANULAR CELL NEUROFIBROMAS: SEPARATION OF NEUROGENOUS TUMORS FROM THE MYOBLASTOMA GROUP. John A. Fust (by invitation) and R. Philip Custer, Philadelphia, Pa.

Abstract. Study of 51 tumors originally diagnosed granular cell myoblastoma at the Army Institute of Pathology and the Presbyterian Hospital disclosed certain structural differences between all but one occurring in the tongue and those located elsewhere. Fifteen of the 16 lingual tumors, if they are true tumors in the neoplastic sense, appeared to arise through alteration of pre-existing voluntary muscle fibers. The 16th was a polypoid tumor bearing no demonstrable relation to muscle and resembling the granular cell tumors found in the skin and subcutaneous tissue at a variety of other sites. In the 35 non-lingual cases we believed it possible to demonstrate a histogenetic relationship to peripheral nerves, perhaps to Schwann cells, which are known to have granular cytoplasm under certain conditions. Large granular cells were frequently found within the perineurium of nerve twigs well beyond the reaches of the tumors, appearing to have developed there rather than having invaded the nerves. Four other tumors showed gradations between the conventional type of neurofibroma and granular cell tumors.

In summarizing this series of 51 cases of granular cell tumors:

1. Those that appear to arise in muscle occurred only in the tongue. Whether they are true neoplasms is not yet clear.
2. Those that appear to arise from nerves may occur in the tongue, but are found mostly elsewhere in skin and subcutaneous tissue. They are true neoplasms.
3. These 2 groups are structurally different, having in common the granular cytoplasm of their component cells.

Two other types of granular cell tumors, not included in this series, may prove to be separate entities. The first is the so-called congenital epulis. The second is found in skeletal muscle, notably in the thighs and buttocks; it differs somewhat in histologic pattern from the lingual lesions and may be more closely related to the conventional rhabdomyoma.

Discussion

(Dr. Jacob M. Ravid, New York, N.Y.) Did any of these granular cell myoblastomas show evidence of malignancy?

(Dr. Fust) All of these tumors appeared to have been cured by simple removal. The only indication of malignancy was the occasional occurrence of carcinoma *in situ* over the tumor. There was one case in the lingual group in which Dr. Custer thought that carcinoma *in situ* was a reasonable diagnosis, and there were 4 among the neurogenous tumors.

HIBERNOMA. REPORT OF CASE. Osborne A. Brines and (by invitation) M. Harvey Johnson, Detroit, Mich.

Abstract. For many years the existence of brown multilobular fat in hibernating mammals and some nonhibernating rodents has been recognized. These masses which are of considerable size have been given a variety of names, the best known of which are hibernating gland and interscapular gland. The hibernating gland is described as being yellowish brown, lobulated, somewhat resembling pancreas or salivary gland, and composed of coarsely granular or finely multilocular cells containing considerable protein but with a smaller fat content than the cells of ordinary fat. Since 1905 approximately 6 neoplasms derived from this structure have been reported in the medical literature. In 1915 Gery reported a case and proposed the name hibernoma, which has been accepted by later contributors. The literature also contains reference to several additional cases which seem to be reasonably authentic but which could be accepted only with reservation. Confusion exists over some "atypical lipomas." Attention is called to the similarity between this tumor and granular cell myoblastoma. It is reasonably possible that some of the former have been mistaken for the latter. Such possible confusion could be eliminated by the employment of fat stains in all granular cell soft tissue tumors. A case of a hibernoma occurring in the right scapular region in an 18-year-old female is reported. The specimen was a nonencapsulated, somewhat flattened tumor measuring 10 by 4 by 2 cm., which was grayish brown and composed of longitudinal parallel bundles having the general appearance of skeletal muscle. There was no recurrence at the end of 1 year.

Discussion

(Dr. Frank Dutra, Cincinnati, Ohio) Is there any relationship between these apparently benign tumors and the malignant liposarcomas? My comment is based on the fact that in benign lipomas foam cells are less common than in those with malignant potentialities.

(Dr. Brines) It is true that foam cells are found in some lipomas. As far as I know, no hibernomas have been reported as malignant or have pursued a malignant clinical course. This patient I have observed only a short time and she has shown no evidence of recurrence. I think it is important, however, that these tumors be differentiated from the so-called atypical lipomas or possibly low-grade liposarcomas, and particularly from granular cell myoblastomas—a mistake which can be avoided very easily.

SCLEROSING LIPOGRANULOMA. Hans F. Smetana and William G. Bernhard, Washington, D.C.

Abstract. The 8 cases presented were characterized by a peculiar tumor-like swelling, persisting from 5 weeks to 12 years, without systemic reaction. This condition occurred in males between the ages of 25 and 43 years and involved the scrotum, spermatic cord, or penis in 6 instances and the buttocks in 2. A history of trauma was recorded in 2 cases, while an inflammatory process of the penis preceded the appearance of the swelling in 2 others. No history of trauma was elicited in the remaining 4 cases.

The clinical picture was that of progressive noninflammatory swelling compatible with neoplasm. In 7 cases a cure was effected by surgical excision, but in one instance the swelling recurred several times despite surgical intervention. The "tumors" received at operation varied from 3 to 8 cm. in diameter and con-

sisted of fatty tissue, with partial substitution by fibrous bands containing cystic spaces.

Histologically, there was replacement of the subcutaneous fat tissue by fat globules of unequal size, separated by fibrous septa which were infiltrated by mononuclear wandering cells and a few eosinophils and polymorphonuclear leukocytes. Many of these fat globules were surrounded by multinucleated foreign body giant cells. The predominant cells were macrophages which had phagocytized fat droplets; small free fat droplets were present also in the fibrous septa and in tissue spaces. Some of the vessels showed perivascular, predominantly lymphocytic, infiltrations. Fat droplets, free as well as surrounded by foreign body giant cells, were seen in perivascular and perineural lymphatics. Occasionally the parenchyma of regional lymph nodes was partly replaced by fat in macrophages, lining cells of sinuses, and foreign body giant cells. Fibrous tissue showing a tendency to hyalinization was present between the fat globules of the subcutis, roughly corresponding in amount to the age of the lesion; in chronic cases there was extreme hyaline scarring with foci of calcification.

Histogenetically, the earliest lesion discernible was focal necrosis of some of the septa of the subcutaneous fat tissue with subsequent confluence of fat droplets to large, irregularly shaped globules. Inflammatory cells appeared and many of the free fat droplets were taken up by macrophages; some of these then migrated into the lymphatics and tissue spaces. Macrophages became so greatly distended by the engulfed fat droplets that their cytoplasm gradually disintegrated, again setting free the fat. By that time, foreign body cells had formed surrounding the fat globules. Neither fatty acid crystals nor soap formation were observed. The size of the globules varied, the larger ones being small spaces, surrounded by numerous multinucleated giant cells. Fibroblasts and connective tissue elements became more and more evident, finally incarcerating the fat globules in scar tissue.

The pathogenesis of this process is not clear. It appears, however, that certain injuries of the subcutaneous fat tissue may cause a profound local disturbance of fat metabolism in some individuals, which leads to liberation of fatty substances acting as foreign bodies, with consequent foreign body reaction. This reaction is similar to that observed in the lungs in cases of lipoid pneumonia and in the pelvic tissues after uterosalpingography with lipiodol. There is also great similarity to the local histologic processes observed in Weber-Christian disease and traumatic fat necrosis of the breast.

Discussion

(Dr. Lester S. King, Chicago, Ill.) I should like to ask what Dr. Smetana considers the relation of this to Weber-Christian disease.

(Dr. Wiley D. Forbus, Durham, N.C.) Dr. Smetana's problem is very interesting to me, especially, because of the possible influence that fat may have over the motility of the reticulo-endothelial cell. We have been working recently on the effects of peanut oil in the lung, and we have found, quite contrary to what Dr. Smetana has seen, that the peanut oil, although phagocytosed, remains in the lung and is not transported to the lymph nodes. I wonder if the fats which were present in Dr. Smetana's materials are more effective in stimulating the movement of phagocytic cells than other forms of fat. In Whipple's disease, about which you will hear in the next paper, the fat appears in a very strange form in macrophages in the intestinal mucosa. That fat stimulates the movement of the macrophages to an extraordinary degree, and you will find in the lymph nodes of the mesentery great quantities of these fat-laden cells, but

the fat now has assumed a new form which provokes a marked reaction exactly like that which Dr. Smetana has shown. I wonder if Dr. Smetana will comment on the stimulative properties of different types of fat.

(Dr. Smetana) I consider the local histologic changes seen in Weber-Christian disease practically identical with those present in sclerosing lipogranuloma. The disease is, of course, differentiated from sclerosing lipogranuloma by its clinical features. In addition, while the lesions in Weber-Christian disease will disappear after some time with local atrophy of the involved fat tissue, we have not seen this course in lipogranuloma.

As to the influence of different fats on the activity of macrophages, I believe the paper by Henry Pinkerton (The reaction to oils and fats in the lung, *Arch. Path.*, 1928, 5, 380-401) deals with this subject. He described the deposition of animal and mineral oils in bronchial lymph nodes after intratracheal instillations, while vegetable oils were not transported to the lymph nodes. This may be related to the absence of specific lipases for mineral and vegetable oils in the animal body. The transportation of fat to the regional lymph nodes in cases of human lipogranuloma is therefore the more remarkable since this phenomenon indicates absence of splitting of autogenous fat. It may be suggested that a subtle chemical change of the subcutaneous fat occurs during its extrusion from the normal fat cells which renders it resistant to splitting; however, in the absence of chemical studies, this can only be assumed.

LIPODYSTROPHY INTESTINALIS (WHIPPLE'S DISEASE). B. Black-Schaffer, and (by invitation) J. P. Hendrix and P. Handler, Durham, N.C.

Abstract. The pathologic anatomy and physiology of lipodystrophy intestinalis (Whipple's disease) is re-examined in the light of 4 new cases. The disease may be recognized by nonlipid macrophagocytosis of the lamina propria of the small intestine and occasionally the proximal colon, lipogranulomatosis of the mesenteric and draining lymph nodes, and the absence of significant evidence of chylous obstruction. Because of the uniform absence of chylous obstruction, the presence of steatorrhea, and poor glucose absorption (glucose tolerance tests) it is postulated that, as in sprue, lipodystrophy intestinalis is produced by a functional defect of the enteric epithelium resulting in decreased ability to absorb fats as well as glucose and possibly proteins.

Lipodystrophy intestinalis differs from sprue in that it is marked by pathognomonic anatomic changes and does not result in the development of macrocytic anemia.

The nature of the nonsudanophilic material in the intestinal lamina propria and the lymph nodes is unknown. As preserved (Kaiserling's solutions), it cannot be identified chemically as lipid.

It is suggested that because of the high incidence of fibrous pericarditis and arthritis that Whipple's disease may be the sequel to an as yet unidentified systemic illness characterized by inflammation of the serous membranes.

Discussion

(Dr. V. H. Cornell, Washington, D.C.) We have had a case very similar to his which I should like to add to Dr. Black-Schaffer's series. I will call attention to two features which he has already demonstrated: one is the nonlipid macrophagocytosis of the intestine by the reticulo-endothelial cells and the other is the tumor-like growth in the lymph nodes. The authors, like myself, were unable to demonstrate the lipids which we expect in Whipple's disease, but we

arrived at a slightly different conclusion, in that they conceived it as an inherent inability of the reticulo-endothelial system to take care of the fat that is brought to it, and therefore the disease is really a reticulo-endotheliosis, in which the cells are biologically deficient.

(Dr. Arthur C. Allen, New York, N.Y.) With regard to Dr. Black-Schaffer's theory of disturbed glucose metabolism as a factor in Whipple's disease, I should like to ask if he has had the opportunity to do histologic phosphatase studies in these cases.

(Dr. Black-Schaffer) We have not done any phosphatase studies in these cases. We should like to point out that phosphorylation is not always an essential step in absorption of carbohydrates. Fourman, utilizing xylose, has published this fact in a recent issue of Clinical Science. I would hesitate to accept Whipple's disease as an example of a primary reticulo-endotheliosis. We believe it to be an example of nonlipid and, in the lymph nodes, lipid phagocytosis, secondary to a functional defect of the enteric epithelium. There does not appear to be any disturbance of the reticulo-endothelial cells.

FACTORS INFLUENCING THE PATHOGENESIS OF EXPERIMENTAL OVARIAN TUMORS IN RATS. G. R. Biskind, and (by invitation) R. Pencharz and M. S. Biskind, San Francisco, Calif., and New York, N.Y.

Abstract. The hormonal imbalance that follows transplantation of an ovary into the spleen of a castrate rat initiates the development of a luteoma through stages of continuous formation and enlargement of corpora lutea. After a prolonged period a granulosa cell tumor appears in the luteoma. These tumors do not appear if the rat is hypophysectomized. In rats that retain one normal ovary and that have the other ovary transplanted to the spleen, the transplanted ovary atrophies and the normal ovary hypertrophies. If the normal ovary is removed after the ovary in the spleen has undergone atrophy, the latter resumes growth and progresses through the stages of continuous luteal formation and enlargement as noted in the original tumors.

Discussion

(Dr. Howard T. Karsner, Cleveland, Ohio) Naturally I am deeply interested in this excellent study. Has time permitted determination of whether these tumors are transplantable?

(Dr. Russell L. Holman, New Orleans, La.) Were any feminizing characteristics associated with these tumors, or couldn't you tell about that?

(Dr. Lester S. King, Chicago, Ill.) Were any vaginal smears made?

(Dr. Antonio Rottino, New York, N.Y.) Is it possible to transplant the ovary of one animal into the spleen of another animal?

(Dr. Biskind) In answer to Dr. Karsner's question, we have some studies on transplantability, but they are not complete. It is extremely difficult to transplant the tumors that develop in rats; however, the tumors that develop in mice are readily transplantable. I have not completed our experiments on anterior chamber transplantations.

The feminizing effect occurred in some granulosa cell tumors. These tumors elaborated so much estrogen that it passed through the liver and produced cornification of the vagina and uterus.

Vaginal smears are performed routinely. In the first period after transplantation the predominant cells were leukocytes, occasionally epithelial cells were

evident. Later, as the granulosa cell tumor is developing, a smear composed of cornified epithelial cells may replace the castrate smear.

The transplantation of the ovary of one animal to the spleen of a castrate rat, male or female, is readily accomplished and all transplants "take."

VIRILIZING HILUS CELL TUMORS OF THE HUMAN OVARY WITH A REVIEW OF OVARIAN HILUS CELLS AND EVIDENCE OF THEIR ANDROGENIC FUNCTION.

William H. Sternberg (by invitation), New Orleans, La.

Abstract. The hilus of the adult human ovary normally contains nests of cells morphologically identical with Leydig cells and whose function is probably androgenic. The morphology of these cells (originally described in detail by Berger) is discussed and their relationship to nonmyelinated nerve and vascular spaces emphasized. Particular stress is placed upon the finding in the cytoplasm of crystalloids of Reinke, structures considered specific for Leydig cells. Two instances of tumors of these cells with masculinizing syndromes are presented. One similar well established case exists in the literature, but probably others have been misdiagnosed. Two additional cases of giant ovaries with stromal hyperplasia and clinical masculinization, in which the hilus cells were increased in number, are presented. There is also evidence that these cells respond to chorionic gonadotrope.

Discussion

(Dr. Jacob M. Ravid, New York, N.Y.) Was any biochemical work done on these tumors, and what specifically is the difference between them and those of the clear cell group which are also virilizing in nature, and for which Rottino and McGrath created the term of "masculinovoblastoma"?

(Dr. A. R. Kantrowitz, Brooklyn, N.Y.) Were any polarizing microscope studies performed on frozen sections of the tumors and normal control ovaries?

(Dr. Sternberg) In answer to Dr. Ravid's first question, not many histochemical studies have been done on this curious group of cells. The Feulgen-Verne reaction is positive as it is in the Leydig cells of the testis. I am not aware of any additional studies of a chemical nature that have been done on these cells. Additional studies are being done.

We feel that this group of virilizing hilus cell tumors is different from the so-called group of masculinovoblastomas. The hilus cell tumors derive from specific cells present in the normal ovarian hilus, which have all the morphologic characteristics of Leydig cells, including the unique crystalloids of Reinke. This is, then, a specific tumor derived from a single cell type normally present in the ovary. I have no doubt that some instances of masculinizing tumors, classified in the literature as masculinovoblastomas, adrenal rest tumors, and luteomas, may have been misdiagnosed and are in reality tumors of this type. I believe that there is such a thing as an adrenal rest tumor but that it is morphologically distinct from hilus cell tumors.

PIGMENTED NEVUS: FACTORS OF AGE AND ANATOMICAL SITE. Herbert Z. Lund and (by invitation) G. Dorr Stobbe, Cleveland, Ohio.

Abstract. To establish a basis of comparison in the study of melanoblastomas, 200 pigmented nevi, selected from four general cutaneous sites, were divided according to age intervals. Lentigo and blue nevi were excluded. Of the features studied only a few can be presented.

Although origin from and relationship to follicles and sweat glands, besides the epidermis, influenced shape, depth, and pigmentation of nevi, it was found that nevi from all sites followed the same general pattern of development. In early life proliferation of cells in the epidermis, dermo-epidermal junction, follicles, and sweat glands is seen and, as a trend, there was diminution in later years both in degree and in percentage of nevi showing it. In successive decades of life the percentages of nevi showing moderate to marked proliferation were: 90, 44, 19, 19, 6, 7, and 0. The last figure represents all cases above 60. There was no abrupt cessation at a given age. The figures indicate either persistence of junctional proliferation from birth or incipience in any of the age intervals. Other histologic features are helpful in the distinction.

Traub and Keil doubt that junctional proliferation is part of the developmental process of benign nevi and give it the special significance of indicating a process which is "potentially malignant (precancerous) whether it arises early or late in life." The above data contradict this. It is part of the developmental process of benign nevi. Although melanoblastoma may have origin in a similar site, such a diagnosis requires other considerations and evidence.

The percentages of nevi in successive decades of life showing any mitotic figures at all were: 20, 12.5, 6, 0, 3, 0, and 0. The last figure represents all cases above 60. Mitosis, always uncommon, was found more often in childhood and youth, and occurred in the junctional as well as in the deeper cells.

As the junctional cells proliferate they migrate from the site of origin and differentiate to the usual "nevus cells." The accumulation of nevus cells separates epidermis from corium. Often proliferation about follicles and sweat glands occurs deeply in the corium. The cells become more fusiform and fibrillar with age. The percentages of nevi in successive decades of life showing much fibrillar proliferation were: 10, 25, 45, 58, 71, 79, and 100. The last figure represents all cases above 60.

Parallel with the increase of fibrils is the appearance of nerve-like elements. The percentages of nevi in successive decades of life showing such were: 0, 0, 13, 14, 26, 48, and 56. The last figure represents all cases above 60. Thus, the "neuro-nevi" are appearances in later stages of development.

It is seen that many of the so-called "types" of nevi are actually nevi in various phases of development. From a consideration of the features discussed, nevi can be classified as young, intermediate, or old.

Although there were variations in certain features of nevi according to anatomical site, this cannot be discussed in this limited presentation.

Discussion

(Dr. Arthur C. Allen, New York, N.Y.) I should like very strongly to endorse Dr. Lund's plea for a re-examination of the whole field of nevi and melanomas. On the other hand, in anticipation of work just completed by Dr. Spitz as well as by myself, and in view of the seriousness of the problem, it might be worth recording some disparity regarding one or two of the basic features which Dr. Lund postulated. It is beginning to be a fairly well known fact that metastasizing melanomas in children are extraordinarily rare; they occur, but they are rare. Nevertheless, as indicated by Dr. Spitz,* the histologic picture of the benign juvenile melanoma may be indistinguishable from those melanomas that kill after puberty. We feel most positively that the so-called junctional change after puberty acquires a peculiar and different significance and virulence that it does not have prior to puberty, and if disregarded, may lead to tragic developments. It is just this kind of junctional change that occurs almost constantly in

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the pigmented lesions of the soles of the feet in adults—a common site of melanomas notwithstanding the virtual absence of the ordinary intradermal nevus in this location.

(Dr. Lund) This presentation is, of course, over-simplified. I could not go into some of the problems which Dr. Allen raised. I will stress, however, that the finding of junctional proliferation alone does not label a nevus as being malignant. We have been disturbed by what appear to be young nevi—in some instances consisting of only junctional and intra-epidermal cells with practically no further differentiation—occurring in persons 20, 30, and 40 years of age. This problem is the subject of further analysis and is one of the reasons we undertook this investigation. Perhaps Dr. Spitz does have the answer and we anticipate the forthcoming publication. The distinction of benign and malignant may be equivocal, but so far in the series of melanoblastomas with metastasis that we are collecting, there have been deviations in the primary lesions from the pattern of development I have described for benign nevi.

The factors of anatomical location could not be given for want of time. The general scheme of development of nevi was similar, however. Neuroid elements, often considered peculiar to the scalp, were found in other anatomical sites, including the distal parts of the extremities.

HISTOGENESIS OF MYOSITIS OSSIFICANS. Lent C. Johnson (by invitation), Washington, D. C.

Abstract. Seventy cases of myositis ossificans were studied for correlation of duration with changes in the histologic picture. A zonal pattern was apparent in almost all lesions and was present invariably during the first 3 months. In the earliest lesions there was an outer zone of damaged muscle, a broad intermediate zone of gelatinous myxomatous tissue, and frequently a central focus of hemorrhage and debris. The intermediate zone quickly differentiated into two layers: The outer layer produced a matrix of chondroid and osteoid character and showed great cellular activity resembling the picture seen in bone sarcoma; later, cellular activity subsided, leaving an outer shell of mature cortex-like bone with a subadjacent layer of cancellous bone. The inner layer of the myxomatous tissue was composed of vascularized sheets of spindle cells with the subsequent development of giant cells, so that histologically this zone simulated a malignant giant cell tumor of bone. Later the giant cells disappeared and the sarcoma-like spindle cells differentiated into an areolar connective tissue which at times suggested fibrous dysplasia. Gradually this connective tissue retracted from the center to leave a cavity which tended to enlarge. The end-stage may be a hollow sphere or tube of bone, lined by velvety, relatively acellular, loose connective tissue.

In general, the histologic appearance of all lesions during the first month strongly suggested a malignant neoplasm. During the second month, about half of the lesions continued to appear malignant, while the rest appeared benign. With very few exceptions, all lesions after the third month could clearly be identified as benign.

A similar zonal pattern with gradual transformation from a process which appeared histologically malignant to one that was clearly benign could be seen in the so-called "subperiosteal giant cell tumor" or "ossifying periosteal hematoma." In the majority of these lesions there was evidence that damaged muscle had participated in the process, thus indicating that many were a form of myositis modified by close association with the periosteum.

A study of myositis ossificans in its earliest manifestations and of the changes in skeletal muscles following crushing injury suggested that the proliferating spindle cells which produced the myxomatous pattern and matrix probably originated from the sarcolemmal sheaths of the partially damaged muscle. In 3 cases, lesions with many of the features of early myositis ossificans tended to behave as sarcoma, with recurrence or apparent invasion. In these cases the zonal pattern of growth was greatly disrupted and the malignant appearance of the cells persisted far beyond the time when the change to a benign form had occurred in the rest of the cases in this study.

SYMPOSIUM ON BONE DISEASES

SKELETAL REFLECTIONS OF DISORDERED METABOLISM.* Henry L. Jaffe, New York, N. Y.

THE SKELETAL CHANGES IN ALBRIGHT'S DISEASE. H. Edward MacMahon, Boston, Mass.

Abstract. In 1937, Albright, Butler, Hampton, and Smith described a syndrome now known as Albright's disease. This was characterized by the presence of multiple bone lesions, areas of skin pigmentation, and precocious puberty in females. They suggested the term "osteitis fibrosa disseminata" to distinguish the skeletal changes in this syndrome from those associated with hyperparathyroidism. A year later Lichtenstein, in stressing the already confused nomenclature of bone diseases, introduced the term "polyostotic fibrous dysplasia" to designate what he considered to be the same bone lesion. Lichtenstein emphasized that the specific changes in the bones can occur alone, and usually constitute the sole disorder, but he admitted that the same bone changes could be found in association with skin pigmentation, precocious puberty, and other abnormalities. Since then the term polyostotic fibrous dysplasia has become modified to "fibrous dysplasia of bone," but in this metamorphosis the original conception as a disease of bone has become greatly expanded, so that cases formerly considered as instances of Albright's syndrome have now come to be regarded as "florid" or "full blown" expressions of this elementary skeletal disorder. Naturally this has created somewhat of a dilemma both in terminology and in interpretation, for it does seem to be somewhat unreasonable to use the same term to denote on the one hand a disease that may be characterized by nothing more than a solitary bone lesion and, on the other hand, to designate a disease that can involve every system in the body—even though a specific skeletal lesion may be common to all cases.

I have had the opportunity to study the skeletal system of a girl, 9 years of age, who died with the clinical diagnosis of Albright's syndrome. Because the study of this child during life by Albright and many others from the age of 6 months to her death was in large measure responsible for the conception of Albright's syndrome, it seems justifiable in a Symposium on Bone Diseases such as this, to report the skeletal findings.

Instead of a single type of skeletal lesion, there were three separate and distinct bone changes. First, and most striking, was the osteitis fibrosa disseminata or fibrous dysplasia that by x-ray and also by autopsy, as far as could be determined, involved every bone in the body. Secondly, there was a disturbance in epiphyseal growth involving both bone and cartilage; and lastly, there was widespread bone atrophy.

* By invitation of the Council.

The first of these, which in some respects was the most striking feature of the entire syndrome, showed many of the gross and histologic features of both fibrous dysplasia and osteitis fibrosa cystica associated with hyperparathyroidism. The bones were deformed, lighter in weight, softer in consistency than normal, and could be easily broken. There were signs of old and more recent fractures. In many bones the normal pattern of cortex and medulla was partially or completely replaced by areas of firm, rubbery, gritty, gray fibrous tissue. The histologic changes in such areas were characterized by a progressive replacement of normal bone and bone marrow by fibrous tissue, cartilage, osteoid tissue, myxomatous tissue, and new bone.

The second type of bone lesion was centered in and about the epiphyseal cartilage. In some areas the epiphyseal lines were nearly normal, but in many places they were ragged and uneven and blunt tongues and long finger-like projections of cartilage extended deeply into the adjacent diaphysis. In other areas segments of epiphysis had completely disappeared, allowing the epiphyseal and diaphyseal bone to fuse prematurely. Still another change involving cartilage was a cessation of skeletal growth with the apposition of well formed bone plates in areas where active endosteal cartilaginous osteogenesis should at her age be taking place.

The third change affecting the skeletal system was characterized by atrophy and osteoporosis. This was most clearly seen in the cortex in areas entirely free of fibrosis. In such fields dilated haversian canals were filled with actively hematopoietic marrow.

Of these three changes, the fibrous dysplasia was by far the most striking. It is of particular interest that there were many histologic features in common with both fibrous dysplasia and hyperparathyroidism. At first, this might suggest a unique condition, but the finding of four enlarged parathyroid glands composed of an almost solid sea of cells, of which many were of the water-clear type, could possibly explain this unusually complex and apparently "combined form" of bone lesion.

Discussion

(Dr. Louis Lichtenstein, New York, N. Y.) There are so few autopsied cases of fibrous dysplasia of bone on record, or of Albright's syndrome, if you will, that this contribution of Dr. MacMahon's is a very important one. It was extremely interesting, therefore, to follow the whole gamut of skeletal changes which he described, though it is a pity that the limitation of time caused him to rush through them so quickly. In the main, the skeletal findings which Dr. MacMahon described are in accord with the changes Dr. Jaffe and I have observed in less severe cases of fibrous dysplasia affecting one, several, or many bones, with or without associated pigmentation and various endocrine disorders. The features suggesting hyperparathyroidism, on the other hand, we have not encountered, but then we have not had the fortunate opportunity of examining any of these extremely severe cases at autopsy. I should like to ask one specific question in regard to the distribution of cartilage foci within the interior of the affected bones: Were they all found in the neighborhood of the plate regions, so one might assume they represent fragments of pinched-off cartilage, or were some of them found well down the marrow cavity, so that one would have to explain them on some other basis, such as an integral feature of the skeletal dysplastic condition? We were already aware of the presence of such cartilage foci when we published our first paper on fibrous dysplasia of bone and, indeed, suggested at that time that this disorder represented a developmental anomaly in a sense related

to skeletal enchondromatosis, and that the two conditions might be regarded as first cousins.

(Dr. Joseph E. Pritchard, Montreal, Canada) Were there any kidney changes or any renal insufficiency in this case?

(Dr. MacMahon) In answer to Dr. Lichtenstein's question about the distribution of cartilage, there were large islands of hyaline cartilage in the immediate vicinity of epiphyseal lines, but there were larger islands far removed from these areas, particularly at sites of earlier fractures. Perhaps most interesting were the islands of cartilage within the substance of the calvarium which, being a membrane bone, is ordinarily free of cartilage. Cartilage adjacent to the epiphysis appeared to be of epiphyseal origin, whereas cartilage forming in the shaft seemed to arise locally, being formed directly by endosteal fibrous tissue or indirectly through the formation of a chondroid mucinous ground substance. The fate of the cartilage was as interesting as its origin. Some islands composed of hyaline cartilage showed no sign of change; some showed maturation, marginal calcification, and enchondral ossification; others showed direct metaplasia to bone and still others showed marginal resorption by growing fibrous tissue and multinucleated chondroclasts.

In answer to Dr. Pritchard's question, the kidneys were well preserved and showed no sign of renal decompensation. Like so many of the organs of this child, the kidneys did show developmental anomalies, but they were relatively insignificant and consisted of a few minute pyramidal cysts.

NEUROFIBROMATOSIS AND ITS RELATIONSHIP TO CERTAIN DISEASES OF BONE.
Ernest Aegerter, Philadelphia, Pa.

Abstract. Neurofibromatosis is a condition characterized by multiple nodules of fibroblastic hyperplasia of nerve supportive tissue. It is variably associated with a number of lesions in bone: scolioses, asymmetric hypertrophy, and focal neurofibromas usually misdiagnosed as bone cysts. It or its associated conditions accompany congenital pseudo-arthritis and fibrous dysplasia in a relatively high incidence.

The contention that fibrous dysplasia is a manifestation of neurofibromatosis has been ably refuted by Jaffe. In this paper the author challenges the widespread belief that congenital pseudo-arthritis is caused by a neurofibroma growing in and destroying bone substance. The clinical behavior of pseudo-arthritis and its gross and microscopic characteristics are cited to show that it is a dysplasia of cortical bone and callus formation.

It is suggested that all the above conditions, including neurofibromatosis, are dysplasias rooted in defective mesenchymal germ plasm. The character of each lesion is apparently conditioned by the modifying influence of its tissue environment. Treatment, especially of pseudo-arthritis, should be designed on this basis. A described successful method appears to substantiate this impression.

PATHOLOGIC CHANGES FOUND IN CURETTINGS FROM THE HEAD OF THE FEMUR IN
31 CASES OF OSTEOCHONDritis DEFORMANS JUVENILIS (LEGG-CALVÉ-PERTHES'
DISEASE). Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. The materials examined were curettings from the head and neck of the femur in 31 cases of Legg-Calvé-Perthes' disease. In 1939 P. B. Steele reported a series of cases on which he had operated to hasten healing in Perthes' disease by opening the hip joint, drilling into the head of the femur, removing the diseased contents, and filling the cavity with curls of bone from the femoral neck. The purpose was to remove débris, support the head with bone grafts, and stimu-

late healing. Thirty-one of 32 clinical cases showed practically the same pathologic changes and varied only in the degree and amount of change. Constant findings were aseptic necrosis or necrobiosis, compression of the various bony elements, and incomplete healing processes which were apparently going on concurrently and to little purpose. The necrosis involved all of the tissues present to varying extents and included destruction of marrow with bony trabeculae recognizable, but without nucleated osteocytes or osteoblasts. Cartilage showed fragmentation, degeneration, and imperfect ossification. The larger bits of cartilage had lost nuclear polarity and the chondrocytes were arranged as constellations of many patterns. Side by side with the degenerated areas were bizarre islets of healing including simple fibrous replacements of marrow stroma which was infiltrated sparsely with lymphocytes and occasional leukocytes. The connective tissue tended to form miniature cysts with foreign body giant cells in their walls. Areas of osteogenesis and osteoclysis were jumbled together and the normal line of ossification of the epiphyseal cartilage was lost. Vascular changes were on the progressive side. Disproportionately larger arterioles with proliferative endarteritis were sometimes seen. The lesions are interpreted by the author as being due primarily to nutritional disturbances associated with vitamin and growth hormone deficiencies. The changes weaken the resistance of the femoral head, and the superimposed minor injuries of weight bearing and frustrated attempts at repair complete the picture.

HISTOCHEMICAL STUDIES ON CARTILAGE AND BONE. Richard H. Follis, Jr., and (by invitation) Morgan Berthrong, Baltimore, Md.

Abstract. Although numerous isolated observations on certain histochemical reactions in cartilage and bone have been recorded, no integrated studies have been carried out. We have felt it desirable to determine the normal histochemical pattern in cartilage and bone in order to prepare for a study of changes in the skeleton produced experimentally by means of vitamins and hormones and bone disease in man.

Observations have been carried out on fresh, free-hand tissue slices and undecalcified sections fixed in various ways. The distribution of the following substances has been studied: cytochrome oxidase, succinic dehydrogenase, citric acid dehydrogenase, alkaline phosphatase, iron, glycogen, mucopolysaccharides, desoxyribose nucleic acid, ribose nucleic acid, neutral fat and ascorbic as well as oxidation-reduction potentials. Certain of the results are as follows. Succinic dehydrogenase is present in cartilage, being particularly prominent in cell nuclei. We have not been able to estimate quantitative differences in relation to maturation of cartilage cells. The osteoblast is rich in cytochrome oxidase as indicated by the nadi reagent. Cartilage does not appear to contain this enzyme. Sections stained by toluidine blue show intense metachromasia of the cartilage matrix material as well as the organic portion of bone, osteoid. Glycogen can be demonstrated in osteoblasts and osteocytes as well as in cartilage cells. The only structures giving a positive Feulgen reaction are the nuclei of cartilage and bone cells. This may be destroyed by incubation with desoxyribonuclease. Colchicine brings out the striking proliferative activity of growing cartilage.

FIBROUS DYSPLASIA OF THE MANDIBLE AND MAXILLA. A. R. Crane and (by invitation) J. R. Wolgamot, Philadelphia, Pa.

Abstract. Eleven cases of monostotic fibrous dysplasia, 6 of the mandible and 5 of the maxilla, have been studied. These show the same feminine predominance as the polyostotic form, 8 occurring in females. Four were in Negroes. Patients

varied from 9 to 56 years of age at the onset. Swelling was the presenting symptom in each case. A history of trauma was given by 4 patients, but this was insignificant in degree. Two cases showed abnormal but slight skin pigmentation. Sexual precocity, genital abnormalities, or precocious skeletal growth were not present. Radiologically, there was focal rarefaction of the mandible or maxilla or increased density in the maxillary sinus. Histologically, all showed fibrous replacement of old bone with membranous new bone formation. Osteoclastic giant cells were present in relation to bone destruction or production, and were a minor part of the picture. Rare, small cystic zones were present in some cases; cartilage was absent. Similar histologic changes were seen in cases of lues, radiculodental cysts, benign giant cell tumors, myositis ossificans of the masseter muscle, and epulides, but these had other identifying features. Ossifying fibroma was not distinguishable from the cases of fibrous dysplasia. Chemical studies (calcium, phosphorus, and alkaline phosphatase) were normal in every case. Patients were treated by curettage or simple re-establishment of normal contour, and are living from a few weeks to 10 years without progression of the abnormality. Lesions of other bones or fractures have not occurred. These findings suggest that there is no line of distinction between monostotic fibrous dysplasia, polyostotic fibrous dysplasia, and ossifying fibroma.

CHONDROMYXOID FIBROMA OF BONE. Louis Lichtenstein, New York, N.Y.

Abstract. This paper deals with a peculiar benign tumor of bone, which seems not to have been generally recognized in the past as a distinctive neoplasm, although it appears likely that single instances of it have been reported as enchondroma or myxoma and their malignant counterparts. Our interpretation of the lesion is that of a peculiarly differentiated, connective tissue tumor exhibiting, in the course of its evolution, certain chondroid and also myxoid traits which hallmark the lesion cytologically. It is composed basically of cells lying loosely in a myxoid intercellular matrix which, as the tumor matures, may undergo substantial collagenization. The tissue of any particular tumor may also come to simulate cartilage tumor tissue in some or many fields; and, in its gross appearance, it likewise bears a certain resemblance to cartilage. The presence of smaller or larger numbers of tumor cells exhibiting nuclear atypism may cause the lesion to appear more ominous than we know it to be, explaining why it may come to be overdiagnosed as a malignant tumor, and particularly, as chondrosarcoma.

Our experience with this tumor to date comprises 8 cases, and we have encountered it thus far only in one or another bone of a lower limb, and specifically, in the femur, the tibia, and some of the foot bones. Within the femur or tibia, the lesion was found consistently in the metaphyseal area adjacent to the knee joint. Most of the patients were adolescents or young adults, though some were older. The lesion, as a rule, evolves slowly and is often of some months' or even a few years' standing before surgical intervention is sought. The roentgenographic picture has a certain distinctiveness, at least when the lesion is in a long bone and has attained appreciable size, although its differentiation at times from bone cyst, enchondroma, or a focus of fibrous dysplasia may be difficult without tissue examination. The tumor is apparently entirely benign and does not tend to recur after mere curettage, even without supplementary radiation. While the tumor is not a particularly common one, its recognition is of some importance in that, pathologically, it may readily be mistaken for a sarcoma, and, as such, treated more radically than is necessary.

Discussion

(Dr. V. H. Cornell, Washington, D.C.) I would like to ask how Dr. Lichtenstein tells them apart.

(Dr. Lichtenstein) Tells what apart?

(Dr. Cornell) The ones that are going to be benign, and those which are going to be malignant. What I mean is this. You have said many of these would be mistakenly diagnosed, and I agree with you. We usually consider myxomatous tissue rather a dangerous thing to play around with. Naturally your history of the cases indicates they are benign. Were there mitoses? Is all the tumor such as we saw on the screen, or is some more abnormal and the cells more irregular? Is there anything to make the differentiation between the benign and the malignant?

(Dr. Joseph E. Pritchard, Montreal, Canada) Is this tumor any different than the common chondromyxoma? Certainly some of them are excentric, but it seems to me that the appearance is very much the same, only some of them are endosteal and some periosteal.

(Dr. Lichtenstein) In reply to Dr. Cornell's question, I venture to state that if you were to see an instance of this particular tumor for the first time or perhaps even for the second time, you might very well fall into the error we ourselves did when we first encountered this lesion. As I remarked, the first two cases which Dr. Jaffe and I encountered were called chondrosarcoma initially. Indeed, it was only on reviewing all the relevant lesions against a background of genuine chondrosarcoma material that we appreciated distinct cytologic differences. Furthermore, we discovered in our follow-ups that the patients in question showed no tendency to local recurrence even after simple curettement. In selecting the photomicrographs I attempted to illustrate various cytologic features of the lesion, and the over-all impression thus created may have been that of a lesion of rather variegated appearance. Actually, the tumor under discussion has a distinctive cytologic picture which is rather easy to recognize if one is familiar with it. To one who is not familiar with it, the cytologic picture may be misleading and create a false impression of malignancy. If this presentation is instrumental in preventing a half dozen or more needless amputations, I think it will have served a useful purpose. We know that the tumor is benign on empirical grounds, for it seems reasonable to assume that when a lesion is treated by simple curettement and does not recur during the ensuing 6 or 7 years, that it is clinically benign, no matter what its cytologic character may be. In other words, we must judge the tumor by what it does, rather than by what it looks like.

As to the question raised by Dr. Pritchard, there are distinct gross and microscopic pathologic differences between the appearance of this tumor and the common garden variety of enchondroma, although it is true that roentgenographicallly one cannot always distinguish them with assurance.

EWING'S TUMOR OF BONE. Horace K. Giffen, Youngstown, Ohio.

Abstract. In 1922 Ewing described a primary malignant bone tumor which is found in children mainly. He called it "diffuse endothelioma of bone." The first evidence of the disease is usually associated with fever, pain, tenderness, and occasionally leukocytosis. The primary site is often in the diaphysis of a long bone. There is marked osteolysis with spread along the shaft, cortical destruction, expansion, and perforation. Through the blood stream other bones, oc-

casional regional lymph nodes, and eventually the lungs may be involved. There is marked temporary response to radiation. Current opinion favors considering these tumors as malignant reticulomas or reticulosarcomas.

Two examples of this clinical entity follow. The first was a 13-year-old boy, who began to complain of pain in the upper right thigh in mid-July of 1945. While playing baseball on August 9th he fractured his femur without direct trauma. Roentgenography and biopsy showed endothelial myeloma. Radiation gave temporary relief, but by January of 1946 headaches had begun. During the following months spread of tumors progressed until his death on September 17, 1946.

The second case was a 16-year-old girl who complained of her left shoulder in May, 1946. For about 2 months a chiropractor treated the shoulder for "dislocations." Admitted to our hospital in mid-July, biopsy and roentgenologic examination revealed endothelial myelomatous involvement of the left scapula. No other bony lesions could be found at that time. But by September 13th there was evidence of metastasis in the 3rd lumbar vertebra. During the following weeks she became emaciated, anemic, febrile, and blind in the left eye. She died on November 11, 1946, or about 6 months after the first symptoms.

Discussion

(Dr. Osborne A. Brines, Detroit, Mich.) Was there any adrenal involvement in either one of these cases?

(Dr. Alfred Angrist, Jamaica, N.Y.) The question of neuroblastoma I think deserves some stressing here, because sometimes the primary can be rather small and still originate from the usual or an unusual site for the neuroblastic tumors or the less differentiated forms of ganglion cell tumors. It has been my experience that though Ewing's tumor is a common surgical diagnosis, this diagnosis is uncommon at autopsy, and this has been true even when the diagnosis has been established by eminent authorities. We have established a rule that the surgical diagnosis of Ewing's tumor in a youngster requires ruling out a neuroblastoma, and in an adult requires ruling out a bronchogenic carcinoma. In every case, and we have had about a dozen in which the diagnosis of Ewing's tumor was made clinically, autopsy showed one or the other of these tumors.

(Dr. Giffen) The first case did show a metastatic lesion in one of the adrenals. It was in the adrenal cortex with no involvement of the medulla. We looked in vain for other lesions of the sympathetic nervous system or any other lesion which might have been the primary tumor. The first case had multiple small lesions in both lungs, small intestine, pancreas, mesenteric lymph nodes, both kidneys, one adrenal cortex, dura, and outer cerebral cortex. The other case showed lesions in the soft tissues of both lungs, pancreas, dura, leptomeninges, and outer cerebrum. I do not feel that in either of these cases there was any evidence of primary tumor elsewhere; we certainly thought of possible primary tumor elsewhere, but we could not find it.

PLASMA CELL MYELOMA ASSOCIATED WITH HIGH CONCENTRATION OF PLASMA LIPOPROTEIN. R. M. Hill (by invitation), R. M. Mulligan and (by invitation) S. G. Dunlop, Denver, Colo.

Abstract. The patient was a white female houseworker, 55 years old, with severe sensitivity to cold for 7 years previous to admission on August 7, 1945. Exposure to cold resulted in severe erythema and urticaria of the exposed parts followed by the development of shallow ulcers.

She sustained a fracture of the left humerus 1 year before admission. Examination disclosed a temperature of 99.6°C ., blue mottling of the fingers and toes, and pain and tenderness on motion of the left shoulder. The urine was negative. Her blood coagulated when withdrawn from a vein into a test tube and reliquefied on warming. Blood hemoglobin was 4 to 5 gm. per cent; erythrocytes, 1,950,000 to 3,250,000 per cmm.; and leukocytes, 6,700 to 7,200 per cmm., with a differential of 30 to 48 per cent segmented neutrophils and 50 to 62 per cent lymphocytes. Serum calcium was 10.4 mg. per 100 cc.; phosphorus, 4.2 to 7.1 mg. per 100 cc.; and alkaline phosphatase, 2.4 Bodansky units. Roentgenograms revealed expansion and destruction of the head of the left humerus. On August 31st incision for biopsy of the lesion in the left humerus was followed by hemorrhage. On September 9th the entire floor of the mouth became so swollen that dyspnea supervened and the patient died with a fever of 107.8°C . at 12:30 p.m.

A sample of freshly drawn heparinized blood, centrifuged at room temperature (25°C .) at 2,000 r.p.m. for 20 minutes, separated into three layers; an upper layer of apparently normal plasma, a middle pearly white layer solidifying on cooling, and a lower layer of cells. In a sample of 7.5 cc., the volumes of these layers were 3 cc., 2.8 cc., and 1.7 cc. The pearly white middle layer (hereinafter referred to as a cryoprotein) was 37 per cent of the volume of the blood and gave the characteristic reactions of a protein. Four subsequent blood samples showed the same phenomenon. After blood transfusion, blood samples failed to show the same change, since the entire plasma solidified on cooling and, by diluting and cooling the plasma, the cryoprotein separated as a flocculent precipitate. On August 11th, total plasma proteins were 7.2 gm. per 100 cc. with albumin 2.2 gm. per 100 cc., and globulin plus fibrinogen 5.0 gm. per 100 cc. Blood nonprotein nitrogen was 29 mg. per 100 cc. and cholesterol 146 mg. per 100 cc. On August 24th, the total plasma proteins were 10 gm. per 100 cc., with albumin 1.8 gm. per 100 cc., globulin 6.5 gm. per 100 cc., and fibrinogen 1.7 gm. per 100 cc. The protein content of the cryoprotein layer was 13.1 gm. per 100 cc. The top layer revealed a total protein of 7.1 gm. per 100 cc., with albumin 2.1 gm. per 100 cc., globulin 4.5 gm. per 100 cc., and fibrinogen 0.5 gm. per 100 cc. On August 28th, following blood transfusion, the total plasma protein was 18.1 gm. per 100 cc., with albumin 2.1 gm. per 100 cc., globulin 9.8 gm. per 100 cc., and fibrinogen 6.2 gm. per 100 cc. At 38°C . the viscosity of the plasma of the patient was 5.3 times that of the average normal, rose rapidly with falling temperature, and reached infinity at 32°C . The cryoprotein was purified by 10 reprecipitations from distilled water, finally brought into aqueous solution, and precipitated by the addition of an equal volume of 95 per cent ethyl alcohol. After standing for 3 days at 4°C . in the icebox, needle-like crystals proved to be cholesterol ester. Microbiologic assay of the amino acids of the purified protein was compared with the analyses of the amino acids in normal plasma globulin and albumin.

Autopsy revealed plasma cell myeloma involving the bone marrow, chiefly of the left humerus, scapula, and clavicle, but also of the ribs, vertebrae, sternum, and skull, the spleen, and a mesenteric lymph node; edema, hemorrhage, and focal atelectasis of the lungs; a gelatinous cast in the bronchus to the lower left lobe; focal acute pharyngitis; clinical Ludwig's angina; and a recent unhealed incision of the left arm. The parathyroid glands, heart, liver, kidneys, brain, and spinal cord were normal. The bone of the head and neck of the humerus was extensively destroyed by massive proliferation of cells having the structure of plasma cells, chiefly in the plasmablast stage. The cells exhibited abundant basophilic cytoplasm and frequently an eccentric position of

the nuclei, which were large and rounded, contained much fine heavily stained chromatin, one or more prominent nucleoli, and numbered one to four to a cell.

BONE TUMORS COMPOSED OF ATYPICAL AMYLOID. William H. Bauer (by invitation) and J. F. Kuzma, St. Louis, Mo., and Milwaukee, Wis.

Abstract. The necropsy of a 59-year-old male who died of purulent meningitis revealed destructive tumors of the sphenoid, the left 9th rib, and the 9th dorsal vertebra. Roentgenographically the other bones failed to show any changes. The clinical record revealed the absence of Bence-Jones proteinuria, hyperproteinuria and albuminuria. The sedimentation rate was normal, peripheral blood appeared unchanged, there was no excessive formation of rouleaux, no "greasiness" of the blood smears, and the calcium-phosphorus metabolism was unaltered. The liver, spleen, kidneys, and intestine microscopically and macroscopically were unchanged.

The lesions of the aforementioned bones consisted of numerous bodies of an atypical amyloid with an abundance of giant cells. Only the sections through the sphenoid contained a scattering of atypical plasma cells and a few lymphocytes. The microscopic findings did not conclusively substantiate the diagnosis of multiple myeloma. The atypical amyloid differed from typical amyloid not only in its distribution, but also by its indistinct reaction to methyl violet and Congo red. Furthermore, it not infrequently displayed a smooth transition into osseous tissue and even appeared more densely calcified than the bone trabeculae. These findings suggest that its chemical composition differed from that of typical amyloid. Microscopically, the predominant formation of the atypical amyloid was by fusion of the protoplasm of degenerated bone marrow cells and giant cells. Secondly, intercellular protoplasm-like globules of unknown origin participated in its deposition. Whether we were dealing with atypical multiple myeloma or the result of a chronic infectious process cannot yet be answered.

* * *

BERYLLIUM PNEUMOCONIOSIS. Walter W. Jetter, Boston, Mass.

Abstract. Beryllium has come into recent importance because of its use in preparing fatigue-resistant copper alloy and fluorescent lamp powder. Aside from an acute and apparently spontaneously subsiding episode of chills and fever similar to "metal fume fever," pulmonary reactions to beryllium may fall into either of the following two types.

The first type of reaction may occur after several weeks or months of exposure to dusts or fumes containing beryllium fluoride or oxyfluoride arising during extraction or processing of the ore. The pathologic lesion in the lungs consists in the activation of alveolar macrophages which then may accumulate in the intrapulmonary air spaces. The usual course is spontaneous remission but occasionally distortion and obliterating fibrosis of lung parenchyma may lead to severe pulmonary insufficiency. The deleterious process has been attributed to the acid fluoride and oxyfluoride radicals rather than to beryllium *per se*.

The second type of pulmonary reaction is a peculiar form of pneumoconiosis, "berylliosis" or "beryllium granulomatosis," which develops insidiously and is chronic in its course. Beryllium has been incriminated in most or nearly all of the cases. In our experience, the disease has occurred among workers exposed to fluorescent lamp powder (zinc manganese beryllium silicate) although similar cases have been reported in the manufacture of beryllium alloys. The lungs in fatal cases have shown extensive nodular and irregular fibrosis. Cor pul-

monale is characteristically present and it is apparent that right heart failure has contributed significantly to death. Microscopic examination indicates that the initial lesion is an intra-alveolar collection of phagocytes. Foreign body giant cells are prominent in this inflammatory process and occasionally they may be seen to contain, or to be in relation to, peculiar basophilic bodies, sometimes referred to as "conch shells." As the granulomatous lesions expand peripherally they tend to merge with one another so that widespread areas may become involved. Concurrent fibroblastic infiltration and resultant scar formation leads to extreme distortion and contraction of contiguous lung tissue. Scarring and hyaline change in a discrete nodule may result in a structure resembling the silicotic nodule. In contrast to silicosis, complicating tuberculous infection has not been observed. The final picture is an extraordinary mixture of active focal and diffuse granulomatous inflammation in combination with dense nodular and irregular fibrosis and hyalinization. The tracheobronchial lymph nodes are enlarged and show varying degrees of active inflammation and connective tissue scarring. Nodes completely replaced by hyalinized fibrous tissue may be encountered. The occurrence of granulomatous lesions in liver and spleen may indicate that the toxic agent or agents are disseminated by the general circulation.

Another prominent feature in beryllium pneumoconiosis is severe, diffuse, obliterating pulmonary endarteritis. The resultant decrease in the capacity of the pulmonary vascular bed may be largely responsible for the occurrence of cor pulmonale and ultimate right heart failure.

Discussion

(Dr. Donald A. Nickerson, Salem, Mass.) These cases are interesting to us, because it was in 1942 that Connolly recognized this condition, and there are now about 60 cases which have roentgenologic and clinical evidence of the disease. Perhaps the most interesting thing about this is that after a new plant was opened the content of beryllium in the phosphor was changed. Up to that time this particular company had used 12.9 per cent beryllium, whereas the new manufacturer used 2.5 per cent. After this change, as far as we know, only 1 or 2 cases have developed.

Another interesting thing is that I have seen 3 cases of injury to the skin. In those sites we have found changes exactly identical with those in the lung, and in those lesions beryllium has been found. Perhaps we will see more of it, because one of these cases was in a young boy who was using a burned-out tube as a baseball bat.

In the cases I have seen the ash content of the lung has shown in all cases levels of 5 μ g. of beryllium per 100 gm. of lung tissue.

(Dr. Jetter) I wish to thank Dr. Nickerson for his interesting comments. The evidence indicates the likelihood of a causal relation in the production of this chronic pulmonary disease by inhalation of beryllium-containing fluorescent powders. Positive proof awaits the production of the disease following experimental exposure in laboratory animals.

ENLARGEMENT OF THE BRONCHIAL ARTERIES AND THEIR ANASTOMOSES WITH THE PULMONARY ARTERIES IN CHRONIC PULMONARY DISEASE. Averill A. Liebow, and (by invitation) Milton R. Hales and Gustaf E. Lindskog, New Haven, Conn.

Abstract. In 14 of 17 lung specimens from patients prepared as casts by the vinylite corrosion technic, great enlargement of the bronchial arteries and numerous anastomoses of these vessels with the pulmonary arteries were observed. The

communications were multiple and usually occurred about the bronchiectatic sacs which involved the fourth order branches of the segmental bronchi, and beyond. In half of the specimens the anastomoses equalled or exceeded a diameter of 1 mm. The enlargement of the bronchial vessels probably occurs in response to the increased demand for oxygenated blood in the granulation tissue during the course of the organizing pneumonia that usually precedes bronchiectasis, and in the hypertrophied muscle and hyperplastic lymphoid tissue that are often observed. The anastomoses may represent persistent communicating channels originating in granulation tissue that receives vessels from both the bronchial and pulmonary arterial systems.

So large and numerous are the anastomoses as to suggest that they have physiologic importance: (a) In shunting blood away from the diseased tissue into relatively intact parenchyma, where the blood pressure is presumably lower; (b) in producing general hypertension in the pulmonary circulation.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder if the authors have had the opportunity to correlate such anastomoses with the state of hypertrophy of the right heart. My second question is fortuitous if the technic of these investigations has been published, but if not, will Dr. Liebow describe it? The preparations are the most exquisite I have seen in a long time.

(Dr. Liebow) We have had only one autopsy specimen from a patient with severe bronchiectasis. This was demonstrated on the screen. In that case there was great enlargement of the right side of the heart, but this was associated not only with bronchiectasis but with a very striking bilateral pulmonary emphysema. One might expect that the burden of the right heart would be increased if there were a diffuse bilateral bronchiectasis. In unilateral bronchiectasis the pressure in the pulmonary artery would be increased only if there were a back flow within the pulmonary artery of the involved side from the region of the anastomoses with the systemic bronchial circulation into the proximal end. Indeed it is possible that this may occur since the anastomoses are so large and so numerous. Catheter studies will have to be done to determine whether or not "back flow" occurs. In the absence of back flow, unilateral bronchiectasis should not produce pulmonary arterial hypertension since the pulmonary capillary bed of one lung is sufficient to accommodate the entire cardiac output without rise in pulmonary arterial pressure, as Courmand has demonstrated.

In answer to Dr. Angrist's second question, the technic has been published in the Bulletin of the International Association of Medical Museums, and there is a demonstration of the method among the exhibits of that Association.

THE RELATIONSHIP OF FIBROCYSTIC DISEASE OF THE PANCREAS TO A DEFICIENCY OF SECRETIN. Archie H. Baggenstoss, and (by invitation) Marschelle H. Power and John H. Grindlay, Rochester, Minn.

Abstract. In a recent study of the pancreas in uremia a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of secretion were observed in approximately 45 per cent of the cases. A high incidence of the same lesion was observed also at necropsy in cases of carcinoma of the stomach, obstruction of the small intestine, chronic ulcerative colitis, and sepsis. It has been suggested by one of us that the following factors are important in the pathogenesis of this lesion: (1) inhibition of the type of pancreatic secretion normally stimulated by secretin, (2) nervous stimulation of the pancreas, leading

to depletion of zymogen granules and formation of a thick, viscid pancreatic juice, (3) dehydration, resulting in inspissation of the juice and the development of intrinsic obstruction, and (4) malnutrition (protein deficiency), resulting in a failure of reparative protein synthesis in the cells of the pancreatic acini.

Because of the similarity of the histologic picture to that observed in the early stages of fibrocystic disease of the pancreas, it was suggested also that a congenital deficiency of secretin was responsible for the latter disease. This hypothesis is based upon the concept first suggested by Blackfan and Wolbach and supported by Farber and Andersen that fibrocystic disease of the pancreas is the result of an abnormally thick and inspissated acinar secretion. If one postulates an inhibition or an absence of stimulation of the pancreas by secretin, the only stimuli to secretion would be nervous and that recently ascribed to pancreozymin. Such an imbalanced stimulation of the pancreas would result in a thick viscid juice which might become inspissated, obstruct the ductules and acini, and lead to atrophy and fibrosis. Stimulation of the pancreas by secretin has been described as causing a flow of alkaline fluid which serves to flush the alveoli, to thin the juice rich in organic material and to sweep it along the ducts. Any satisfactory explanation of the pathogenesis of fibrocystic disease of the pancreas must also explain the disturbances which occasionally occur in the secretions of the liver and intestines. There is good evidence that, normally, secretin stimulates the secretion of bile and the succus entericus. If stimulation by secretin did not occur, the obstruction of the small bile ducts and intestinal glands in these cases might also be explained as the result of the production of an abnormally thick inspissated secretion.

In order to test this hypothesis it was necessary to determine (1) if secretin could be obtained from specimens of the duodenum and small intestines at autopsy, and (2) if it were absent in patients dying of fibrocystic disease of the pancreas. Up to the present we have been able to extract secretin (S) according to the methods outlined by Ivy and his co-workers from specimens of the upper part of the intestinal tract of 17 of 18 adults. The single failure occurred in a case of chronic intestinal obstruction of 4 weeks' duration. The extraction of secretin in the other cases was successful whether it was carried out immediately or after the specimen had been frozen for varying intervals of time up to 39 days. Secretin could be extracted from specimens kept unfrozen as long as 14 hours post-mortem. We have been able to extract secretin from the upper part of the intestinal tract of all children studied except premature and newborn infants and one child with fibrocystic disease of the pancreas. The specimen from the child with fibrocystic disease was sent to us through the courtesy of Dr. J. P. Simonds and Dr. Philip-born of Chicago. We are hoping to obtain a sufficient number of specimens to test our hypothesis thoroughly before arriving at any conclusions. For the present we should like to suggest that fibrocystic disease of the pancreas is the result of a congenital deficiency of secretin. This deficiency may be the result of a congenital absence (relative or complete), a defect in the mechanism of its release, or its destruction by abnormal amounts of secretinase. This preliminary report of our work is concerned only with the first possibility.

Discussion

(Dr. William R. Platt, Louisville, Ky.) Does this deficiency of secretin have anything to do with the production of meconium ileus in the newborn?

(Dr. Betty B. Geren, St Louis, Mo.) How does Dr. Baggenstoss postulate that the lack of secretin would produce changes in the salivary glands or bronchial mucous glands as seen in cases of fibrocystic disease?

(Dr. Baggenstoss) I think the fact that meconium ileus occurs also indicates

a deficiency in secretin. Normally, secretin stimulates the intestinal glands and increases the flow of succus entericus. If secretin were absent, it might explain the inspissated meconium that occurs in those cases. It does not explain the changes that have been described in the salivary glands. We did not observe these changes, and a number of other investigators have not been able to find them. If dilatation of salivary gland acini is an essential part of the disease, deficiency of secretin does not explain it. The changes in the bronchial glands we have ascribed to pulmonary infection.

PRODUCTION OF UNILATERAL ULCERATIVE PULMONARY PHTHISIS BY QUANTITATIVE NATURAL AIRBORNE CONTAGION.* Max B. Lurie and (by invitation) Samuel Abramson, Philadelphia, Pa.

Abstract. A fine suspension of tubercle bacilli, free from clumps, is sprayed with compressed air through a specially designed nozzle. The large particles settle out quickly. The invisible droplet nuclei are sucked into a pipe 16 feet long through a chamber in which rabbits are exposed, by the draft action of a flame at the bottom of a chimney devised by Wells. The infected air, after its incineration in the hot flame, is drawn to the outside by a fan. The concentration of the tubercle bacilli in the air respired by the rabbits is determined culturally by a modified Wells air centrifuge. Since the volume of air breathed by the rabbits in a given time can be determined, and since the number of bacillary units in a given volume of air is known, the number of bacilli to which the rabbits were exposed can also be estimated. When rabbits were killed immediately after exposure it was found that the number of bacilli cultured from the lungs corresponds closely to the number of bacilli estimated to have been inhaled.

The number of primary tubercles developed in the lungs is to some degree proportional to the number of bacilli in the air respired by the rabbits. However, there is great variation between individual rabbits inhaling the same infected air both as to the number of tubercles developed and their progression.

Inbred rabbits of high genetic resistance to tuberculosis were immunized with heat-killed tubercle bacilli and exposed to the inhalation of about 50 droplet nuclei of the virulent bovine bacilli. In some rabbits there was no tuberculosis at autopsy. Others acquired nonprogressive encapsulated cavities in one or both lungs, while still others developed a unilateral ulcerative phthisis. There was no dissemination of the disease by lymphogenous or hematogenous routes. The disease thus acquired strikingly resembled human ulcerative phthisis of the reinfection type.

Discussion

(Dr. Murray D. Angevine, Madison, Wis.) I should like to ask Dr. Lurie about the incidence of involvement of the liver and spleen in this group, or was the kidney representative of the dissemination?

(Dr. Kornel L. Terplan, Buffalo, N.Y.) I have a brief comment on the interesting experiments of Dr. Lurie. From his findings no conclusions should be drawn as to the presence or absence of tuberculous lesions in lymph nodes draining the site of reinfection tuberculosis in man. Even if these lymph nodes are grossly not conspicuously enlarged, as a rule, in microscopic examination, recent tubercles are found in the bronchomediastinal lymph nodes. Such findings sometimes are very marked, especially in older individuals, obviously in connection with considerable loss of their resistance against tuberculosis. Occasionally in such cases, even gross enlargement is seen, with more or less marked caseation of the lymph nodes

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draining pulmonary areas, the site of a progressive reinfection tuberculosis.

(Dr. Alfred Angrist, Jamaica, N.Y.) Will Dr. Lurie comment on what the photographs seem to show, as to apical localization in these rabbits, contrary to the usual site of localization in rabbits?

(Dr. Stanley H. Durlacher, Edgewood, Md.) How far down the respiratory tract do these aerosol particles extend?

(Dr. Lurie) In answer to Dr. Angevine's question, I can say that tuberculosis in the liver or spleen of rabbits under these conditions rarely occurs. Occasionally, microscopic tubercles may be seen in these organs in rabbits with a long-standing disease, but visible, macroscopic tubercles are extremely rare, whereas tuberculosis of the kidney nearly always occurs.

As to the question of Dr. Terplan, I must say that these lymph nodes have not been investigated completely. In a number of animals in which the lymph nodes were studied microscopically no tubercles were seen, but in other cases they were found.

As to the question of apical tuberculosis, the primary tubercle in the lung of rabbits may occur anywhere in the lung; however, rarely, if ever, are apical localizations seen. The lower lobe sometimes is affected, but in my experience the upper lobes of both lungs are frequently the site of the primary lesion.

In reply to the last question, as to how far down in the lungs the tubercle bacilli reach, I may say that the most frequent localization of these tubercles is subpleural, therefore it must be assumed that the bacilli penetrate to the terminal divisions of the respiratory passages.

TUBERCULOSIS IN RABBITS INDUCED BY DROPLET NUCLEI INFECTION: RESPONSE TO INITIAL INFECTION AND TO REINFECTION. H. L. Ratcliffe and (by invitation) W. F. Wells, Philadelphia, Pa.

Abstract. When rabbits were caused to inhale virulent bovine tubercle bacilli as separated cells in droplet nuclei, the initial tubercles developed at a highly uniform rate and followed a highly uniform pattern for a period of about 5 weeks after infection. Thereafter, progress of the disease varied with the animal and was proportional to the number of bacilli contained in the lesions. Hence it was concluded that rabbits do not differ in their inherent resistance to this organism, but differ widely in their ability to acquire resistance.

As judged by differences in the rate of development of initial tubercles after the fifth week, resistance developed slowly and the rate of its development varied widely. Experiments on inhaled reinfection show, however, that demonstrable levels of resistance were developed in all animals within 2 weeks after initial inhaled infection by small numbers of organisms. Within 5 weeks after small initial infection, resistance reached levels which inhibited reinfection by virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei. In so far as could be determined by these experiments, the basic effect of acquired resistance of rabbits to this organism was the inhibition of its multiplication.

Discussion

(Dr. Max B. Lurie, Philadelphia, Pa.) I am glad to hear that this study on inhalation primary infection and reinfection confirms our work reported in a series of papers in *The Journal of Experimental Medicine* from 1928 to 1942. These have shown that in immunized animals the tubercle bacilli of reinfection are destroyed immediately if given in small numbers. If large numbers are employed the bacilli of reinfection fail to grow. We have also shown that the increased

destruction of the bacilli of reinfection is a primary function of the mononuclear phagocytes themselves. Humoral factors also play a rôle. You may remember that we placed mononuclears derived from normal and immunized animals, that had phagocytized the tubercle bacilli, *in vitro*, into the anterior chambers of the eyes of the same rabbits, and that we found that the cells derived from the normal animal permitted the free growth of tubercle bacilli, whereas the cells derived from immunized animals inhibited this growth, even though these cells had been transferred to a normal environment. Furthermore, this difference in the fate of the bacilli in normal and immunized animals has also been confirmed by Karl Jensen from Copenhagen, who also used inhalation methods, though his work was not as quantitative as that of Ratcliffe and Wells. He found that the chief difference between behavior of tubercle bacilli of reinfection as distinguished from bacilli of primary infection was that the former failed to multiply whereas the latter grew freely. I repeat that I am happy to learn that our work was again confirmed.

(Dr. Ratcliffe) In so far as we could determine from this material, the effect of the monocytes on the growth of the bacilli is not very striking. The most rapid points of growth of the bacilli seem to be outside of the cells, in the necrotic center of the tubercle, even when the tubercle is advanced, and in reinfection, if any growth takes place, it seems to take place outside of the monocytes, or outside of the other cells of the tubercle. It seems to me one might speculate as to the effect of antibodies rather than that of the cells themselves in this inhibition of the multiplication of the organisms.

PATHOGENICITY STUDIES OF TUBERCLE BACILLI, TYPE AVIUM, FROM A HUMAN INFECTION. William H. Feldman, Dorothy Hutchinson (by invitation), Virginia Schwarting (by invitation) and A. G. Karlson, Rochester, Minn., and Oak Terrace, Minn.

Abstract. Definitely proved progressive tuberculous infections in human beings are exceedingly rare. In this case report impressive evidence was obtained that supports the belief that a pulmonary infection in a 2-year-old child was due to tubercle bacilli of avian origin. The child, a member of a farm family, was ill with a pulmonary disease of undetermined etiology for several months prior to a diagnosis of *pulmonary* tuberculosis of the upper left lobe. On admission to the sanatorium a gastric lavage specimen was obtained which yielded a culture of acid-fast bacilli. The culture was unlike that of typical human tubercle bacilli and subsequently extensive studies of pathogenicity were done. The results indicate definitely that the organism studied is a typical avian tubercle bacillus. A survey of the child's environment before entering the sanatorium revealed several significant facts in support of the possibility of the farm animals being the source of the infection. The animals were tested with avian tuberculin with the following results: Among 430 chickens, 217 (50.4 per cent) reacted positively; among 6 swine, 2 reacted positively; and among 33 cattle, 10 reacted positively. None of the mammals reacted to mammalian tuberculin. Extensive lesions were present in most of the chickens examined at necropsy and cultures of avian tubercle bacilli were obtained from the birds examined. In addition the child frequently played with the chickens and often handled eggs. Tuberculosis was not present in the immediate family consisting of the parents and 4 other children, aged 6 months to 10 years. Under sanatorium care the child's condition has improved continuously. She will probably be released within the next few months.

Discussion

(Dr. Joseph D. Aronson, Philadelphia, Pa.) I should like to know whether the spleen was enlarged in this child, and whether blood cultures were made from this case. This is a thoroughly worked-up case and most interesting, because there is so much in the literature on avian tuberculosis in man which is questionable.

(Dr. Frank Dutra, Cincinnati, Ohio) In July last year 12 men who had been cleaning pigeon dung out of an attic became ill with what seemed to be an acute pneumonitis which required quite a long time to resolve. In December one of these men died of coronary thrombosis, and an autopsy was made. The lungs were grossly normal, but microscopically throughout the lungs there were a fair number of miliary lesions which strongly resembled tubercles. There were various stages, and many of them had gone on to complete fibrosis. Some similar lesions were found in the bronchopulmonary lymph nodes. The possibility that these men had been infected with avian tuberculosis was raised. Unfortunately, the body had been embalmed when the case came to my attention, so that no cultures could be made. Attempts were made by Dr. Albert Sabin to get some avian tuberculin from Dr. Feldman, and I believe tests are going to be made on the other 11 people who had been ill. We do know that there have been three other epidemics of similar pneumonitis in people working with pigeons in the United States since 1940, and I would like to know what Dr. Feldman thinks about the possibility of these cases being avian tuberculosis. Perhaps Dr. Feldman has some information as to whether avian tuberculin tests were done on the people in the other groups who were working with pigeons and developed pneumonitis.

(Dr. John R. Schenken, Omaha, Neb.) Were the infected chickens sensitive to human tuberculin?

(Dr. Stuart Mudd, Philadelphia, Pa., addressing Dr. Dutra) Did you have tests made on these pigeons for ornithosis?

(Dr. Dutra) Dr. Sabin did carry them out and they were negative. The possibility that the man who died might have had psittacosis also seems to be ruled out by the nature of the lesions.

(Dr. Feldman) I am sorry, Dr. Aronson, I cannot tell you about the possible enlargement of the spleen of this child. Dr. Hutchinson did not record that in her abstract. It is my impression that no attempts were made to culture tubercle bacilli from the blood.

As to whether pigeons might or might not be responsible for the respiratory infection in the human beings mentioned by Dr. Dutra, I think one guess is as good as another. I think we should first establish in those pigeons that remain whether they have tuberculosis. If they have not, this information will save a lot of subsequent work.

(Dr. Dutra) All that has been recovered from the pigeons is *Toxoplasma*.

(Dr. Feldman) As to whether tuberculous chickens react to human tuberculin, we have found that a very small percentage do react.

SEROLOGIC REACTIONS OF PATIENTS WITH SARCOIDOSIS TO ANTIGENS OF MYCOBACTERIUM TUBERCULOSIS. William H. Carnes and (by invitation) Sidney Raffel, Stanford University, Calif.

Abstract. The cause of sarcoidosis has not been established. Although it is rarely claimed that tubercle bacilli can be identified in relation to the lesions of the disease and the great majority of the patients fail to react to tuberculin,

a number of investigators adhere to the theory of tuberculous origin. The incidence of complement-fixing antibodies in the sera of 22 cases of sarcoidosis has been investigated with the use of a high molecular weight protein of the tubercle bacillus. More than half the cases were tested also with several other types of antigen including whole and defatted bacilli, protein, lipid, and carbohydrate preparations. The diagnosis was supported in each instance by biopsy. The patients were 19 to 65 years of age (mean age 28 years). Tuberculin tests were reported in 20 of the cases, 4 of which were positive to doses of 0.1 or 1.0 mg. Portions of the biopsy specimens of 16 cases were inoculated into guinea-pigs, all of which proved negative for tuberculosis. Altogether, 6 cases (27.3 per cent) gave positive serologic reactions with one or more antigens. No positive reactions were obtained with carbohydrate antigen in these cases or any of the controls. For comparison with the cases of sarcoidosis, a group of 26 cases of active tuberculosis, bacteriologically verified, was tested similarly. Altogether 16 of these (61.5 per cent) gave positive fixation reactions. Only one case of tuberculosis had a negative tuberculin test and this patient's serum had the highest titer of antibody (160 units per cc.) found in any serum. Sixty-seven per cent of the positive sera of tuberculous patients had titers of 40 units of antibody per cc. or higher with the protein antigens, whereas only 38 per cent of the positive sera of patients with sarcoidosis had titers of 40 units per cc., and none were higher. A further comparison was made with two groups of preclinical medical students recently tested with tuberculin. Of 30 students positive to the first test dose of PPD, but having no clinical or radiologic evidence of active tuberculosis, 10 (33.3 per cent) gave positive serologic reactions. None of a group of 29 students, negative to the second test dose of PPD, gave positive serologic reactions. These results lend no support to the theory that sarcoidosis is due to the tubercle bacillus. The incidence of positive serologic reactions to antigens of *Myco. tuberculosis* found in cases of sarcoidosis may reasonably be explained by previous unrelated tuberculous infection in a portion of the cases.

Discussion

(Dr. A. M. Pappenheimer, Boston, Mass.) We have studied a case in which tuberculosis had supervened on an initial sarcoidosis, and it was interesting to find in the lungs and elsewhere the two types of lesions coexistent, the typical sarcoid tubercles without caseation and without bacilli side by side with large caseating tuberculous lesions. It seems to me that this argues against the idea that sarcoidosis represents an altered reactivity of the tissues to the tubercle bacillus.

(Dr. Donald A. Nickerson, Salem, Mass.) Were skin tests done on these patients using as antigen emulsified material from the skin lesions?

(Dr. Joseph D. Aronson, Philadelphia, Pa.) It might be of interest to Dr. Carnes to know that we have vaccinated a number of cases of sarcoid intracutaneously with BCG vaccine. While these cases developed a local tubercle they still remained tuberculin negative. It would be interesting to have Dr. Carnes follow the cases serologically after injecting BCG to see what would happen.

(Dr. Wiley D. Forbus, Durham, N.C.) I hope that what I say will not be regarded as facetious in any respect, but may I raise a question? Would it not be a good idea for some ingenious man, and we must have lots of them, to attack the problem of sarcoidosis from a little different point of view? Instead of proceeding on the theory that it must or must not be tuberculosis, why not adopt the view that it may be any one of a number of other granulomatous

diseases about which we know little at the present time? It would seem to me that study of the problem of the possible relation of sarcoidosis to tuberculosis has now reached the stage that the study of the problem of the possible identity of Hodgkin's disease and tuberculosis long ago reached. Some ingenious young man might take another tack and help us along faster with the problem of the etiology of sarcoid. Certainly, Dr. Carnes has now exhausted one, and perhaps the last, of the possibilities of identifying the disease with tuberculosis.

(Dr. Carnes) I have also seen what Dr. Pappenheimer referred to, that is, the coexistence of typical caseous tuberculosis and sarcoidosis, and I think that my reaction is the same as his, namely, that it is further evidence that the sarcoid reaction is not on the basis of a peculiarity of the patient, as has been suggested, which would account for a different type of reaction to the same etiologic agent.

We have tried a good many patients with lymph nodes, not skin, Dr. Nickerson, testing them in a manner analogous to the Frei test. The emulsion was made in the way the old Frei antigen was made. Although there have been frequent positive reactions reported from the Scandinavian countries, we have not been able to confirm them, but we are still working on it, and have gotten some of the antigen from Dr. Danbolt in Oslo.

In view of Dr. Aronson's statement about the failure of patients with sarcoid who were inoculated with BCG to develop a positive tuberculin test, I think it would be very interesting to find what happens serologically to these patients in comparison with a group of nonsarcoid cases. We have not done this.

Dr. Forbus has pointed out that many etiologic agents will produce a lesion which is essentially like that of sarcoidosis, and that gives us a great deal of difficulty in determining whether there is still another unknown etiologic agent at the basis of the group of cases which we call sarcoidosis. The reasons are too numerous to try to be given right now, but a good many people still believe that there is an etiologic agent or a pathologic and clinical entity in this group of cases of sarcoidosis, in spite of the fact that there are a number of other agents which will simulate the pathologic lesion.

THE MICROSCOPIC STUDY OF LYMPHOID TISSUE IN THE PANCREAS AND ITS RELATION TO LYMPHOMATOSIS IN CHICKENS. Alfred M. Lucas and (by invitation) Eugene F. Oakberg, East Lansing, Mich.

Abstract. Not received.

THE PRODUCTION OF HEMOSIDEROSIS OF THE LIVER IN THE RAT BY DIETARY MEANS. T. D. Kinney, and (by invitation) D. M. Hegsted and C. L. Finch, Cleveland, Ohio, and Boston, Mass.

Abstract. Groups of adult rats were placed on 4 different diets. Control diet 1 consisted of purina dog chow alone, and control diet 2 was made up of purina dog chow plus 2 per cent ferric citrate. Diet 3 contained 80 per cent corn grits and 20 per cent lard. Diet 4 was made up of 98 per cent of diet 3 to which 2 per cent ferric citrate had been added. The animals were fed the diets *ad libitum* for periods of from 7 to 32 days and sacrificed. Both control groups did well. The animals on the corn grits and lard diet lost weight but, in the animals receiving this diet plus iron, weight loss was more precipitate and severe. The differences in the values for the iron content of the livers were striking. In group I they ranged between 7.3 to 13.24 mg. per 100 gm. of liver; group II, 8.5 to 15.6 mg.; group III, 5.4 to 30.1 mg.; and group IV, 35.5 to

93.6 mg. There was no significant variation in the serum iron levels between groups I, II, and III. This was true as well for determinations of the iron-binding capacity of the serum. However, there was a marked increase in the serum levels for the animals in group IV. At the same time the iron-binding capacity levels fell to zero in all but one animal.

Microscopic examination of the livers in group IV showed marked deposition of hemosiderin in the Kupffer cells and in the liver cells about the portal areas. Increased iron was present also in the bone marrow. This was not the case in any of the animals in the other 3 groups.

The mucosal block which has been postulated as the mechanism for controlling iron absorption in proportion to body needs has been overcome in the animals fed the corn grits diet.

Discussion

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask if control studies were made restricting the growth on purina with or without iron. I think it is well recognized that if you restrict the growth of animals by one means or another one can produce hemosiderosis of the spleen and liver. The explanation is probably that if you restrict the growth of the animal there is no necessity for as much iron to make hemoglobin as with the normal rate of growth. I would predict if you restrict the growth of the control animals you will probably get the same result in these as in your experimental animals.

(Dr. Russell L. Holman, New Orleans, La.) I should like to ask if Dr. Kinney has any direct data incriminating corn grits rather than some other ingredient of the diet, such as lard.

(Dr. Joseph J. Lalich, Madison, Wis.) Have you analyzed these organs to find out whether or not they contain ferritin?

(Dr. H. Edward MacMahon, Boston, Mass.) Was there any suggestion of early cirrhosis in any of the livers?

(Dr. Kinney) This paper really represents what we consider a preliminary report of a group of studies on the subject. Subsequent papers will report the work still under way.

We thought of the matter of growth as one of the possible causes of the deposition of hemosiderin, and we were also aware of Dr. Follis' paper on pyridoxine-deficient animals in which hemosiderin was deposited in the livers and spleens of these animals. As I remember Dr. Follis' paper, the animals were on the diets for at least 60 days or more. In our animals iron absorption takes place very rapidly as illustrated by the animal receiving corn grits diet and iron in which there was a marked elevation of the liver iron after 7 days. Further, in another group of experiments not reported today, serum iron levels were determined in fasting animals given single doses of iron mixed with the control and corn grits diets. When the iron was given with purina alone the serum iron showed only a slight initial rise, but when the iron was given mixed with the corn grits diet there was a slight initial rise followed by a prolonged rise. We felt then that these changes occurred too rapidly to be due to starvation alone.

The corn grits in the diet is not responsible, because animals fed rice or white bread instead of the corn grits show the same deposition of iron.

The livers, spleens, and other organs have not been analyzed for ferritin. There was no evidence of cirrhosis in the livers of these animals. In this connection it should be pointed out that the animals were on the diets for relatively short periods of time, usually no longer than 33 days, and although there was consider-

able fat visible in the livers no scarring was found. This is a severe diet and the animals do poorly and do not live much longer than 5 or 6 weeks. The diets were supplemented in various ways in an effort to determine the factor or factors responsible for the excessive absorption of iron. Casein was only slightly effective, while a complete salt mixture was more effective. The addition of phosphate salts to the diet greatly reduced, but did not completely prevent, the rise in the iron content of the liver.

HEMOGLOBINURIA (BLACKWATER FEVER) IN THE MONKEY WITH A CONSIDERATION OF THE DISEASE IN MAN.* R. H. Rigdon, Galveston, Texas.

Abstract. Hemoglobinuria occurred in 10 monkeys infected with *Plasmodium knowlesi*. The pathogenesis is discussed from the standpoint of an excessive amount of pigment in the blood stream which cannot be removed by the reticulo-endothelial system since the latter is already filled with malarial pigment. The excess of hemoglobin pigments, therefore, is filtered through the glomeruli. The tubular degeneration results from the acidosis that accompanies the disease and is not the result of the casts that form in the lumina of the tubules. The oliguria apparently may result from the shock which is the result of the disease rather than as the result of a mechanical blockage of the tubules by the precipitated hemoglobin.

Discussion

(Dr. Joseph F. McManus, Birmingham, Ala.) Have you estimated the non-protein nitrogen or blood urea in these animals?

(Dr. Joseph J. Lalich, Madison, Wis.) Have any blood pressure studies been done on these animals?

(Dr. Rigdon) We did not make any observations on these animals with regard to the retention of nonprotein nitrogen. There are other observations in the literature that would indicate that such does occur.

With regard to the blood pressure in these animals, we did not take it. This is only a part of a study in malaria in which we feel that severe anemia and anoxemia do lead to shock. We feel that these animals show evidence of shock because they respond so nicely to the transfusion of blood and the giving of oxygen. A low blood pressure may be the basis for the decrease in the amount of urine, or the anuria that may accompany blackwater fever. I think that decreased filtration rate is more important in the production of anuria than mechanical blockage of the collecting tubules by casts.

STUDIES ON THE PATHOGENESIS OF EXPERIMENTAL HEMOGLOBINURIC NEPHROSIS IN RABBITS WITH SPECIAL REFERENCE TO THE LATE MANIFESTATIONS.* Joseph J. Lalich (by invitation), Madison, Wis.

Abstract. Experimental hemoglobinuric nephrosis was consistently produced in rabbits by withholding water and food for 3 days prior to the intravenous injection of 1.8 gm. per kg. of homologous hemoglobin. In those rabbits which survived the acute phase of hemoglobinuric nephrosis, a nephrectomy was performed 13 to 17 days following the initial injection of hemoglobin. The rabbits were killed 34 to 116 days after the surgical operation, thus permitting microscopic examination of early and late manifestations of this disease in kidney sections of 12 rabbits.

In the surviving rabbits in the early phase, the total cast number varied from 4

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

to 188 in 10 low-power fields. Associated with the precipitation of hemoglobin there was tubular dilatation with flattening of the epithelial cells. There were also local areas of vacuolization of epithelial cells in the proximal convoluted tubules and in Henle's loops. During this period the rabbits manifested transient elevations of nonprotein nitrogen ranging from 50 to 250 mg. per cent. Following the nephrectomy the rabbits continued to eat and gain weight. Examination of kidney sections removed at autopsy demonstrated the transient nature of hemoglobinuric nephrosis when the animals do not die during the acute phase. In 7 rabbits no pigment casts were found in one longitudinal section. In 5 the total cast count varied from 1 to 5 in 10 low-power fields. Tubular dilatation and degeneration of epithelial cells was no longer evident.

After an animal survives the acute phase of hemoglobinuric nephrosis, death does not occur. Rabbits with extensive deposition of pigment, tubular dilatation, and epithelial degeneration are able to tolerate still further reduction of functioning renal tissue by nephrectomy.

Discussion

(Dr. Bernard Black-Schaffer, Durham, N.C.) I should like to ask whether kidney function tests were carried out at the time of nephrectomy in animals 5, 7, and 10, in order to obtain some idea of the functional potentialities of the one remaining kidney, which probably suffered the same severe changes as were demonstrated in the photographs of the excised kidneys.

(Dr. Virgil H. Moon, Philadelphia, Pa.) This presentation is in a field which is closely related to the one in which we have been deeply interested, that is, the changes occurring in the kidney, morphologically and functionally, incident to shock from various causes. There is disagreement among observers as to where these changes occur, whether they are limited to one portion of the nephron or whether they affect all portions of the nephron rather uniformly. These experiments are of similar type to those we have made, and probably the mechanism of effects is not very different. I should like to ask Dr. Lalich if in his examinations he noted the location of degenerative changes in one portion or another of the nephron.

(Dr. Lalich) The only functional studies done on these animals were plasma nonprotein nitrogen determinations, and, of course, under the conditions of this experiment practically all of the animals manifested some rise of nonprotein nitrogen, sometimes to as high as 260, with a subsequent return to normal levels. I should state that these operations were done during the period of declining nonprotein nitrogen. In most instances the nonprotein nitrogen at the time of surgery was slightly above normal levels, or within the normal range.

In answer to Dr. Moon's question, the majority of casts accumulated in the distal convoluted tubules, then proximal to that we found the dilatation, if it occurs. Dilatation does not necessarily have to occur. The degenerative changes which appear occur in the loops of Henle and the proximal convoluted tubules.

HYPERSPLENIC EXTRACTS. AN EVALUATION OF THEIR EFFECTS ON THE HEMATOPOIETIC ORGANS OF MICE. William R. Platt, Louisville, Ky.

Abstract. Acetone extracts of spleens removed from normal, thrombocytopenic, and neutropenic patients were prepared according to the original method of Troland and Lee. After the completion of control studies, intraperitoneal injections were made into 100 mice of three different strains (Swiss, A, and C57). Observations were then made on the peripheral blood, femoral marrow, lungs, liver, spleen, and kidneys. A discussion of similar experimental attempts by

other workers, an evaluation of the results obtained, and finally the possible clinical applications of such splenic extracts are related in detail in the complete report.

DISSEMINATED ARTERIOLAR AND CAPILLARY PLATELET THROMBOSES. Ira Gore (by invitation) and Nathan B. Friedman, Washington, D.C.

Abstract. Since Baehr, Klemperer, and Schifrin described 4 cases of disseminated arteriolar and capillary platelet thrombosis in 1936, 15 cases have been reported in the literature. There has been considerable speculation regarding the basic mechanism involved. Although a few authors have postulated a primary endothelial lesion, such a premise has been minimized by a few and denied by others. Five cases of this disease were in the files of the Army Institute of Pathology and through the courtesy of Dr. Klemperer and Dr. Schlesinger, slides of 3 previously reported cases were made available for comparison. The findings in these cases indicated a definite vascular lesion of a type previously undescribed as the exciting cause of the syndrome. This early lesion is not often found, since the prompt action of the blood-clotting mechanism results in the formation of the fully developed lesion by the time of death. The latter has been described in the literature and its widely disseminated distribution in arterioles and capillaries gives the disease its name.

The vascular lesion to which the process is attributed consists of the accumulation of hyaline material beneath the endothelium of an occasional capillary or between the endothelium and muscularis of an arteriole. Neither the subendothelial reticulum nor the elastica of the arteriole is involved primarily. In fact, a splitting of the reticulum fibers is regarded as evidence of the formation of the lesion within the wall of the vessel. With the Schiff periodic acid staining reaction the hyaline material takes a bright red hue which contrasts sharply with the yellow color assumed by the remainder of the tissue. The process is a segmental one and many arterioles and capillaries do not exhibit it. In focal areas swelling occurs, evidently by imbibition from the plasma, to form a nodule which protrudes both into the lumen, carrying the overlying endothelium with it, and away from it, causing a defect in the vessel wall. Progressive swelling leads to rupture of the overlying endothelium and provides the focal injury responsible for the platelet thrombus. It seems reasonable to assume that swelling occurs rapidly, for otherwise the well known capacity of the endothelium to proliferate would prevent rupture. It is postulated that the lesion represents a defect of the intercellular endothelial cement substance which has recently been shown to be largely responsible for the permeability of capillaries.

In the earliest of the thrombotic lesions, a bimorphic character is evident. The peripheral portion is composed of the amorphous hyaline material originating from the lesion of the vessel wall; the luminal portion is granular and composed of aggregated platelets. Endothelial proliferative reaction which is initially absent, develops promptly from the intact endothelium at the margins of the initiating thrombus. There is a striking tendency for the thrombi to propagate along the length of the vessel and in many such instances the endothelial growth forms a sleeve-like process investing the thrombus. By contrast, the cells lining the lumen of the vessel containing the lesion are unaltered, a point which is indicative of the focal origin of the investing endothelium. The bulk of the thrombi seen ordinarily in the tissues consist of transections of such propagated thrombi.

The mode of organization provides further support for the primacy of the vascular injury. The endothelial proliferation is clearly an effort to re-establish the integrity of the vascular lining and is manifested first by growth over the

surface of the lesion. When this has been accomplished, there is secondary invasion of the thrombus. Organization does not occur from the base of the adherent thrombus as would be expected if the lining endothelium at that point were previously intact.

Discussion

(Dr. James F. Rinehart, San Francisco, Calif.) Is this a disease in itself, or are these cases of lupus? There is a great similarity, I believe, in certain of the lesions with those seen in disseminated lupus. Is this a separate picture, or is it related to disseminated lupus?

(Dr. Paul Klemperer, New York, N.Y.) I would like to answer Dr. Rinehart's question rather than to discuss the paper, although there is much to be said about it. I hope Dr. Gore will forgive me for replying to Dr. Rinehart. I think there is no question whatsoever of this peculiar condition and lupus being different. One of the points that might be deceptive is that in the original reports in 1925 and 1935 the patients were women, later on cases were reported in men. Except for the fact that there are vascular lesions in both diseases, there is no similarity.

(Dr. Bernard Black-Schaffer, Durham, N.C.) Siegmund described a lesion very similar which has been called in the German literature "Siegmund's nodes." He found them in cases of acute infectious disease. I wonder if the authors have compared this to their lesions.

(Dr. H. Edward MacMahon, Boston, Mass.) In the pictures of this disease that we have been shown today, there appear to be two separate and distinct vascular changes. In one of these the basement membrane of small vessels shows simply fusiform or nodular swelling; in the other, the endothelium is injured or lost and the intima shows a platelet and fibrin deposition. Were these supposed to show two stages of the same process or two distinctly different manifestations of the same disease?

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether fat stains were done on the early lesions. I think, upon comparing them with other findings, as in the Kimmelstiel-Wilson lesions of the glomeruli in diabetic patients, that they may be found positive.

(Dr. Louis Lichtenstein, New York, N.Y.) My question has to some extent been anticipated by that of Dr. Black-Schaffer. I would like to ask Dr. Gore whether there was anything distinctive about the clinical picture of the cases he presented.

(Dr. Gore) I would like to thank Dr. Klemperer for answering Dr. Rinehart's question, and I fully endorse his stand. I do not see any similarity between this condition and disseminated lupus.

In regard to Dr. Black-Schaffer's question, I am not familiar with Siegmund's article. I have not seen similar vascular changes in other conditions. This is the first time I have seen this particular lesion.

The question of Dr. MacMahon I believe is hard to answer morphologically, but Chambers, observing capillaries directly under controlled conditions, has shown that damage to the endothelial cement substance results in softening and there is a dropping off of material into the blood stream so that the lesion of the vessel wall, once the endothelium is ruptured, does not persist. Ordinarily, in tissue sections, too, we see cross sections of the propagated thrombus much more frequently than the focal point from which it arose.

In reply to the question concerning fat stains, we have not done any fat stains on the initial lesion. However, from their staining characteristics I would not suspect fat to be present.

Clinically, these patients have a characteristic clinical course featured by vague

and poorly defined prodromal symptoms, a rapidly progressive anemia, purpura, and thrombocytopenia. Invariably severe nonlocalizing neurologic manifestations occur before death. There does not appear to be a clinical common denominator as an etiologic or predisposing factor.

PLATELET THROMBOSIS IN HUMAN HEMOSTASIS: A HISTOLOGIC STUDY OF SKIN WOUNDS IN NORMAL AND IN PURPURIC INDIVIDUALS. Howard D. Zucker (by invitation), New York, N.Y.

Abstract. The histologic appearance of serial sections of skin puncture wounds, obtained for biopsy from 10 to 20 minutes after injury, was studied with the purpose of investigating the mechanism of hemostasis in normal and purpuric individuals. Hemorrhage from severed muscular vessels up to $250\ \mu$ in diameter is normally arrested by platelet thrombi formed at the site of vascular injury. Platelet thrombi do not form in severed true capillaries. Fibrin is normally seen within the wound tract; it is not seen in the lumen of cut vessels. In idiopathic thrombocytopenic purpura, platelet thrombosis is never seen. Fibrin formation may occur in wounds in idiopathic purpura; its appearance may be delayed for at least 15 minutes. Without platelet thrombosis, fibrin formation within the wound is inadequate to arrest hemorrhage from muscular vessels within 3 minutes. The similarity of the histologic appearance of human puncture wounds to that described after experimental vascular injury in other mammals suggests considerable similarity in mammalian hemostatic mechanisms.

Discussion

(Dr. Joseph Tannenberg, Batavia, N.Y.) I am very much interested in this paper, since I conducted experiments between 1925 and 1930 on animals in which the walls of the small arteries and capillaries could be made visible and microscopically studied during the time of hemostasis. In these experiments I found that the segmentary contraction of the small arterioles and what we called with Ricker and others stasis in the capillaries, *i.e.*, a conglutination of the erythrocytes, were the most important factors in bringing about stoppage of the bleeding. In addition to this the pressure on the veins of the hemorrhage into the tissue seemed to be an important factor. A developing thrombosis and fibrin formation in the bleeding wound, and particularly in the torn wall of small veins, were considered major contributory factors of hemostasis. The last factors may be absent in individuals with thrombopenia and purpura and account for the modification of hemostasis.

(Dr. Zucker) I am familiar with Dr. Tannenberg's papers. In so far as contraction of the arterioles or of any of the muscular vessels is concerned, I am sure that it is an important factor in hemostasis. On the basis of what I have seen in my sections, I would lay primary stress, if anywhere, on the platelets, but certainly the muscular factors are exceedingly important. As far as the conglutination of the red blood cells is concerned, that is something which has not been seen in any of my controls nor in any purpuric sections, and I cannot make further comment on it.

THE INCIDENCE OF RHEUMATIC HEART DISEASE IN INDIVIDUALS WITH CONGENITAL MALFORMATIONS OF THE HEART. S. H. Durlacher and (by invitation) Emil C. Beyer, Edgewood, Md., and New York, N.Y.

Abstract. Consecutive autopsies to the number of 7925, performed in the Department of Pathology, Yale University School of Medicine, were analyzed. There were 6,853 cases over 2 years of age, the age of the youngest rheumatic indi-

vidual. Five hundred and nine hearts showed anatomic lesions of rheumatic heart disease and of these, 43 had active rheumatic myocarditis at the time of death. Among 55 cases with congenital malformations of the heart there were 22 with rheumatic stigmata, and of these, 4 had active rheumatic myocarditis at the time of death. The rheumatic rate among cases with congenital malformations is statistically significantly higher than in the group without congenital lesions even when the rheumatic rate in the latter group is corrected for the age distribution of the former. Rheumatic lesions occurred in 4 of 6 instances of interauricular septal defect, in 2 of 4 instances of tetralogy of Fallot, in 2 of 4 cases of interventricular septal defect, in 1 of 3 cases of persistent truncus arteriosus, in 2 of 6 cases of patent ductus arteriosus, and in 11 of 29 instances of minor malformations. Active rheumatic myocarditis occurred in 2 instances of interventricular defect, 1 case of tetralogy of Fallot, and 1 case with bicuspid pulmonic valves.

Discussion

(Dr. Jesse E. Edwards, Rochester, Minn.) This presentation comes with some surprise to me in that it has been my personal experience that rheumatic heart disease is not unduly associated with congenital malformations of the heart. On the other hand, when one examines hearts from cases of congenital malformations it has been rather common to find that there are localized areas in the endocardium in which there is fibrosis. The fibrosis may be diffuse in one chamber, or it may be localized, and there may be some thickening of the valve leaflets. Most of these endocardial reactions, I believe, are secondary to mechanical alterations on the basis of the congenital malformation. For example, in patent ductus arteriosus it is common, almost without exception, to find the endocardium on the left side of the heart, particularly, showing considerable fibrous thickening with collagen and elastic tissue. My reaction is that the dilatation of the chambers of the left side results in the production of connective tissue in the endocardium, a production which is on the basis of a mechanical rather than an inflammatory change. The same holds true of a defect of the atrial septum. One frequently finds that the leaflets of the mitral valve are thickened, but when one examines those valves carefully, one usually does not find changes in the chordae; they are not fused, shortened, or thickened, and again I believe that dilatation of the atrium, which will cause stretching of the valvular tissue, results in the production of fibrous tissue. In defects of the interventricular septum there may exist localized endocardial thickening which probably can be explained on the basis of mechanical trauma by eddies of blood. It is my opinion, therefore, that in evaluation of cases of congenital heart disease, considerable caution should be exercised before one says rheumatic fever is present, since mechanical factors alone may account for certain endocardial changes. Microscopic evidence of rheumatic carditis is, of course, conclusive.

(Dr. William H. Carnes, Baltimore, Md.) Did you find very many instances of bland vegetations on the valve leaflets, particularly on the right side of the heart with pulmonic stenosis, such as I have encountered, and find frequently commented on in reports of these cases? Were the vegetations of this type which you saw on the valves regarded as being rheumatic vegetations?

(Dr. Alfred Angrist, Jamaica, N.Y.) What percentage of the hearts showed active rheumatic lesions in the myocardium, and did one see active rheumatic verrucae? My own experience parallels that of the discussers, that the association is uncommon. The fact that some of the localized lesions on the valve have mimicked the findings in rheumatic distortion may well represent congenital defects of the valve structures, associated with congenital deformity, because often in newborn congenital hearts you see similar gross distortions of the valve.

(Dr. Durlacher) I agree with many of the things Dr. Edwards said. I was surprised in this analysis to find the high incidence of rheumatic heart disease among patients with congenital heart disease over the age of 2 because I did not expect it. We had a peculiar coincidence when we began operating on cases of tetralogy of Fallot, and one patient died with an acute rheumatic myocarditis. That was the start of the analysis. I was very cautious about interpreting the results when scars were the only criterion for the diagnosis of rheumatic heart disease, and for that reason I separated what I called the active and the inactive cases and subjected them to separate statistical analysis. I do not know that perivascular scarring is any more reliable as a diagnostic criterion for rheumatic heart disease than endocardial scars and neither are reliable for diagnosis. The value of the interpretation of these depends on the ability of the men performing the autopsy. For this reason I tried to get away from that objection by taking only the major valvular deformities, such as were shown here, as criteria, and only cases with such deformities were included. The analysis of the acute cases leaves, I think, no question. I grant the number is small, only 4, but there were 4 of 55 cases of congenital cardiac disease, an incidence of 7 per cent, which is statistically higher than the rheumatic rate in the noncongenital group.

Bland vegetations were encountered frequently in many of the cases, but I was well aware that thrombi on the valves and mural endocardium are quite common in congenital malformations, and they were not used as criteria for rheumatic heart disease.

In answer to the question about the number of active cases, we had 7 per cent active rheumatic carditis of 55 cases of congenital heart disease living over the age of 2.

The question of valvular deformities found in newborns did not enter this series as the analysis included only cases over the age of 2 years and these newborn deformities fell out.

UNEXPECTED DEATH IN CHILDREN WITH RHEUMATIC HEART DISEASE. T. R. Hamilton, and (by invitation) W. A. Tanner, E. M. Pebley, Kansas City, Kansas, and G. S. Voorhees, Leavenworth, Kansas.

Abstract. This report cites 4 instances of unexpected death in children in whom rheumatic heart disease was the pathologic diagnosis. The ages at the time of death were 15, 11, 4, and 2½ years in this group of 2 boys and 2 girls, of whom 2 were white and 2 Negro.

The most dramatic instance was that of a 15-year-old white boy who dropped dead at the blackboard at school. A sclerosing type of lesion involving smaller branches of the coronary arteries was rather marked in this case. There was a history of four rheumatic episodes over a period of 7½ years under medical management. The second case was that of an 11-year-old colored girl found dead in bed on the fourth day of hospitalization. This was shortly after she complained of pain in the chest along with nausea and abdominal distress. She had had her initial rheumatic episode only 4 months before admission and had experienced precordial pain during the last 3 months. Adhesive pericarditis was marked in both of these cases with synechia cordis in the latter instance. In that case a narrow ostium of the left coronary artery was noted. Pancarditis was marked in these enlarged hearts which weighed 660 and 380 gm. respectively. The valvular lesions were not stenosing.

The two younger children were not known to have had rheumatic fever clinically. A white girl, age 2 years and 8 months, died suddenly during morning

care on the first day of hospitalization. This occurred shortly after she had complained of pain in the chest. The fourth child, a 4-year-old colored boy, suddenly collapsed while at play and died in the emergency room of the hospital. Pathologic consideration of these last 2 cases centered on cardiac enlargement. In the 4-year-old child the heart weighed 150 gm., which is three times normal size, but in the 2-year-old child there was no accurate weight. Anitschkow myocytes appeared most strikingly proliferative in vascular areas, particularly along a delicate verrucous margin of valvular endocardium in the youngest child. Pneumonitis was a feature in these 2 cases.

The most striking coronary lesion was noted in the case of the oldest boy; however, he had had fairly persistent cardiac arrhythmia with auricular fibrillation. A narrow coronary ostium appeared to be significant in the case of the older girl. In 3 of the cases pericarditis was particularly striking. Myocardial involvement was a prominent feature common to all.

Discussion

(Dr. Jacob Werne, Jamacia, N.Y.) Most of the pathological conditions which are responsible for death may be associated at one time or another with sudden death. There are certain conditions associated with sudden death which are frequently overlooked unless specific search is made. I refer here to the frequency with which upper respiratory infections are responsible for death in early life. For the discovery of these conditions at autopsy, it is important that the neck organs, mastoids, and middle ears be examined particularly. The presence of lesions in these areas may often explain why subjects with other conditions such as rheumatic heart disease die sooner and more suddenly than might otherwise be expected. With regard to the patient dying 10 days after vaccination, the possibility of post-vaccinal encephalitis must be considered.

(Dr. Arthur C. Allen, New York, N.Y.) I should like to mention that Aschoff bodies of rheumatic myocarditis have been found in a number of instances at the Army Institute of Pathology in previously healthy people who died sudden traumatic deaths. This fact does not negate Dr. Hamilton's findings, but it does introduce a statistical nicety.

(Dr. Virgil H. Moon, Philadelphia, Pa.) Many of those who die of unexplained causes, or of inadequate causes, have been found to have hypoplasia of the adrenal cortex and have lymphoid hyperplasia associated with that. I should like to ask the authors whether examination for these features has been made.

(Dr. Hamilton) The neck organs were not commented upon in all cases. In the last 2 cases pneumonitis was a feature, and it was interpreted as of rheumatic type. There was some evidence of decompensation in the second case, that of the girl who died after angina of 3 months' duration. She had chronic passive congestion of the liver and some congestion of the lungs.

In regard to Dr. Werne's question about the possibility of lesions in the brain following vaccination in case 4, it is unfortunate that examination of the brain was not allowed. There was no evidence of embolism in these cases, which is important, particularly because in the series of Stroud and Twaddle on 15 years' observation of rheumatic heart disease in children, of the 9 with sudden death 5 were under 16 years old, and there was embolism in the majority of these cases. We did not observe any indications of embolic phenomena in our cases.

In regard to Dr. Allen's comment, I think that it is of major interest in sudden death that Dr. Moritz, at the Army Institute of Pathology, analyzed 40,000 cases and found 5 in which there was evidence of rheumatic heart disease. That was an older group, and I did not mention it in this brief report for that reason.

In reply to Dr. Moon's question, we did not observe lymphoid hyperplasia or adrenal hypoplasia in this series.

THE FATE OF BLOOD INJECTED INTO THE ARTERIAL MEDIA. William B. Wartman, Chicago, Ill.

Abstract. These experiments, which have for their purpose the study of the fate of blood injected into the arterial media, were undertaken in order to determine whether or not hemorrhage into the media of an artery plays a part in the initiation of arteriosclerosis or of dissecting aneurysm. Eight dogs were used and a total of 26 hematomas was produced in 17 arteries. Blood from the same animal was injected into the wall of the common carotid and femoral arteries and abdominal aorta. Because of the extreme thinness of the normal canine intima it was found to be impossible to inject blood into it so that only medial hematomas were produced. The results were the same in all vessels and no difference was noted between venous and arterial blood. Study of hematomas at various ages from 3 to 352 days showed that the blood was removed from the media by gradual destruction of red blood cells accompanied by liberation of pigment and mild inflammation. Medial necrosis was present during the first 8 days, but was not observed after this time. The red blood cells disappeared between the 15th and 24th days. Hemosiderin pigment was observed first after 8 days and in most instances persisted for as long as 61 days, but in 2 cases it was found at the end of 392 days. Medial scars were first seen at the end of 48 days. They were composed of collagenous connective tissue and contained capillaries which usually disappeared between 61 and 169 days, although in 2 cases they were observed after 392 days. Moderate fibrosis and hyalinization of the intima occurred occasionally. In no case was there atheroma formation, intimal arteriosclerosis, or aneurysm formation, and no hemorrhage occurred from the new capillaries which grew in the hematomas. The whole process appeared to be one of organization of the hematomas, resulting in the medial scar or in the restitution of the arterial wall. The lesion was apparently self-limited and did not progress either to arteriosclerosis or dissecting aneurysm.

Discussion

(Dr. S. H. Durlacher, Edgewood, Md.) I find it interesting that there were no phagocytes filled with lipids in the media of these vessels. The injection of blood into the peritoneal cavity is followed by the accumulation of large phagocytes which stain with sudan III. I wonder if any of these were found.

(Dr. Wartman) We did occasionally find foreign body giant cells, but no phagocytes containing lipids.

PATHOLOGY OF AMINO ACID DEFICIENCY IN RATS. I. PHENYLALANINE DEFICIENCY.

R. L. Ferguson and (by invitation) Charles Schwartz, Vermillion, S.D.

Abstract. Weanling male rats were fed a completely synthetic diet for periods up to 28 days. Rats of corresponding ages were fed the same diet from which the essential amino acid, phenylalanine, was omitted. Control groups were carried along on a similar diet in which the amino acid mixture was replaced by a corresponding amount of vitamin-free casein, and on a diet in which the amino acid mixture was replaced by $2\frac{1}{2}$ times its weight of vitamin-free casein. All rats were weighed periodically and a record of food consumption was kept.

At intervals rats from each group were sacrificed and sections of all tissues and organs were taken for complete histologic study. The results to date indicate that in those rats fed the diet deficient in phenylalanine, there was a marked retardation of spermatogenesis.

COLITIS IN THE FOLIC ACID-DEFICIENT MONKEY WITH NOTES ON SIMILARITIES TO ULCERATIVE COLITIS IN MAN. James F. Rinehart and (by invitation) Louis D. Greenberg, San Francisco, Calif.

Abstract. Progress in nutritional research has been rapid. One of the most important advances was the development of a synthetic diet adequate for evaluation of a single deficiency in the rhesus monkey. With the conviction that the nutritional requirements of this primate would most closely approximate those of man, we have undertaken systematic studies of deficiency states in this species.

This report is concerned with our observations on folic acid deficiency. In the rather extensive literature on folic acid deficiency there is a surprising lack of detailed study of pathologic changes. Most of the experiments have been concerned with its influence on blood formation. When folic acid is removed from the diet, the animals ordinarily gain weight and remain active for 2 or 3 weeks. At this time they begin to lose weight. Usually in 4 to 5 weeks they develop diarrhea and become less active. Ulceration at the gum margin may develop and the weakness and the diarrhea become progressively more severe.

Our studies on the blood are in essential agreement with those previously reported. The animals develop moderate anemia and leukopenia. Administration of folic acid causes a reticulocyte response, rise in the white blood cell count, and improvement in the gums, bowel function, and general condition. During deficiency the polymorphonuclear leukocytes show characteristic degenerative changes. They become larger, the neutrophilic granules disappear, and bluish bodies are seen in the cytoplasm which is often vacuolated.

We have previously called attention to the characteristic and usually severe lesions which develop in the colon. All of the animals developed diarrhea and of the 7 animals examined post-mortem, 6 showed a structural colitis. In the earlier phases small irregular ulcers with purulent exudate appear in the congested and edematous mucosa. In time the lesions become more extensive. Cystic dilatation is seen in mucosal glands which are lined by atrophic and degenerating epithelial cells. Normal mucous secretory activity appears to be reduced and many of the cells show hyperchromatic nuclei. By others, colitis in folic acid deficiency has been ascribed to a concurrent bacillary dysentery developing in a weakened animal. By stool cultures on 6 of our animals, the Flexner dysentery bacillus was isolated from 2. The others failed to show pathogenic organisms. Our evidence indicates that folic acid deficiency specifically lowers the resistance of the colon to invasion by pathogens and that an ulcerative colitis may develop as a part of the deficiency disease even in the absence of pathogenic organisms. The lesions bear a very close resemblance to those of ulcerative colitis as it is encountered in man. It is of particular interest that one animal which we maintained in a state of chronic deficiency for 23 months showed practically complete loss of mucosa and a rigid, thick-walled colon with a narrow lumen corresponding to the lesion seen in the late stages of ulcerative colitis.

Our observations suggest that folic acid deficiency may be a factor in the pathogenesis of ulcerative colitis in man. This possibility is certainly deserving of critical clinical study.

Discussion

(Dr. John H. Fisher, London, Ont.) I should like to ask Dr. Rinehart if he saw any evidence in his animals of cirrhosis of the liver. We have recently seen a human case of long-standing, but relatively mild, chronic ulcerative colitis, in which an advanced cirrhosis of the liver was present.

(Dr. Russell L. Holman, New Orleans, La.) I should like to ask two questions.

First, have you any data on therapeutic tests, and second, have you observed any changes in other mucosae, for example, that of the respiratory tract? If you have not noticed any difference in the mucosa in other parts of the body, do you have any idea why it is present only in the colon?

(Dr. Jacob Werne, Jamaica, N.Y.) I wonder whether the sera of these animals from which no enteric pathogens were cultured were examined for antibodies.

(Dr. Rinehart) This group of animals did not have cirrhotic change in the liver. Some of the animals subjected to pyridoxine deficiency which we have studied have shown such changes. In respect to therapeutic trials, these we have not made.

With regard to other mucosal changes: we have found none, other than that described in the oral mucous membrane. I am unable to understand the pathogenesis of the lesion in the colon at this time.

I appreciate the suggestion of examination of the serum for antibodies to dysentery bacilli and will include it if further study is made. Our study was not directed toward colitis; initially it was simply a study of folic acid deficiency. I might point out one thing I neglected to say, that other animals subjected to equal degrees of inanition have not shown colitis, with one exception. A pyridoxine-deficient animal did develop an ulcerative lesion in the colon.

PATHOGENESIS OF CRYPTOCOCCIC (TORULA) MENINGITIS. Kornel Terplan, Buffalo, N.Y.

Abstract. In the majority of the post-mortem reports on cryptococcic infection recorded in the literature, the central nervous system was the only organ involved. All of the recorded, so-called pulmonary cases (about 20 altogether) were, possibly with a rare exception, associated with the well known lesions in the central nervous system, especially in their coverings. In most of the cases of Torula meningitis, including the generalized types with lesions in various organs and with miliary changes in the lungs, no morphologic data are available which could point to the respiratory tract as the portal of entry.

The anatomic and histologic findings of an otherwise typical case of cryptococcic meningo-encephalitis observed in a 28-year-old Indian laborer, who was admitted to a tuberculosis sanatorium for suspected tuberculous meningitis, are presented. Spinal fluid samples yielded abundant masses of *Cryptococcus neoformans*, the pathogenicity of which was established by animal inoculation. Death occurred 5 months after the initial symptoms of meningitis. Apart from the characteristic findings of gelatinous meningitis with small cysts in the cortex and wart-like granulomas in the dura, extending into the left gasserian ganglion, a well encapsulated, bean-sized nodule, found in a subpleural position in the right lower lobe, measured 11 by 6 mm. It resembled in consistency and color a typical primary caseated tuberculous focus. Histologic analysis revealed a well encapsulated area, including within the alveolar framework colony-like masses of yeast cells with well preserved mucinous capsules. The histologic structure of the tissue surrounding this focus pointed to simple capsule formation by collagenous fibers blending with the thickened pleura and containing numerous capillary vessels, moderate infiltrations of lymphocytes, and an occasional giant cell of the foreign body type. Part of the adjacent lung tissue was atelectatic. There were no other lesions produced by this fungus, either in the lung and its regional lymph nodes, or in any other organ, including the mediastinal pleura. The paranasal sinuses and middle ears were uninvolved. This primary tubercle-like lesion caused by the yeast cells was the only one which could have served as the source for the secondary hematogenous spread to the leptomeninges.

Discussion

(Dr. Wiley D. Forbus, Durham, N.C.) I should like to add a word in support of what Dr. Terplan has said about the primary pulmonary origin of the infection in cryptococcic meningitis. We have recently had an experience which helped us a good deal in this connection. In our case we found a primary lesion comparable in every respect to that which you have seen on the screen. The primary lesion lay immediately beneath the pleura. It had ulcerated through the pleura and spilled its contents into the pleural space, resulting in an extensive invasion of the pleural lymphatics and those of the parenchyma of the lung. This was followed by widespread dissemination, but there was no involvement of the meninges. After that experience we made a study of all of our other cases of cryptococcosis, including those in which no lesion had been found anywhere except in the meninges. We returned to the gross material, made careful serial sections of the lungs, and found the primary focus in every case as an old fibrous or caseous nodule. This observation, of course, does not rule out certain other possible primary foci. Those who are familiar with the problem will recall the paper published by Urbach and Zach in which there was extensive cryptococcic infection of the mouth. Obviously, such a lesion might serve as the point from which the organisms were disseminated to the internal organs.

(Dr. Terplan) I would like to add that a few rare observations have been recorded with unusual portals of entry of the cryptococcic infection. The case to which Dr. Forbus referred is apparently one of these; and there were a few others, one with a deep ulcer in the tongue, one with a lesion in the skin (from a razor blade), and also one where the rectum was thought to be the site of the original infection. The fact, however, remains, that in the majority of the cases reported in the literature only meningeal and cerebral involvement has been observed and the lesion or lesions in the lung were not detected.

THE PATHOLOGY OF HERPES SIMPLEX ENCEPHALITIS IN MAN, WITH A REPORT OF THREE CASES. Webb Haymaker, Washington, D.C.

Abstract. In 3 proved cases of herpes simplex encephalitis, previously reported in abbreviated form, the pathologic changes were very similar. The most striking lesions were in the cerebral cortex: they consisted mainly of massive necrosis, replacement of superficial lamina by large mononuclear and gitter cells, and perivascular invasion of parenchyma by mononuclear cells; in 2 of the cases there were myriad neuronophagic nodules. Topographic study on the available material disclosed that lesions were most severe in the cerebral cortex, hippocampal formation, subcortical white matter, claustrum and some of the basal olfactory nuclei, and affected to much less degree were the striatum, thalamus, and cerebellum. The pallidum, amygdala and spinal cord were spared. Type "A" intranuclear inclusion bodies were found in profusion in ganglion cells and oligodendroglia of the cortex and subcortical white matter, and to a lesser degree elsewhere. They stained most satisfactorily with hematoxylin and eosin.

Discussion

(Dr. Margaret G. Smith, St. Louis, Mo.) I was very much interested in Dr. Haymaker's presentation. The histologic lesions are much like those which we have seen in the one case which has been reported and in two others which have not been reported. I think there are several points of interest about these cases. All of the patients have been quite young people, one of ours being a month-old infant, the second a 14-year-old boy, and the third a 17-year-old girl. The possible route of entry of this virus into the central nervous system has

interested me, and also what relation there may be between the development of encephalitis and the individual's state of immunity. The last two of the cases that we have studied have been very interesting in that the lesions were confined almost entirely to the olfactory areas of the brain, suggesting that the olfactory pathway had been the route of entry of the virus into the central nervous system. In the case of the young infant we could obtain no serum from the mother to test for antibody for the herpes virus. The other two patients, as far as we could learn from their families, had never had herpetic skin lesions.

(Dr. Haymaker) As regards the occurrence of herpetic lesions over the preceding years in our cases, there is no history of such available.

The basal olfactory localization of the lesions referred to by Dr. Smith is of considerable interest, inasmuch as in one of our cases the initial symptom was that of "peculiar smells"; these persisted for 3 or 4 days, and then the patient complained of experiencing "peculiar tastes." Since olfactory sensation may be represented, in part at least, in the pyriform area, and since this region, together with the anterior perforated substance and nucleus accumbens septi, were markedly affected in our case, the olfactory pathways may well have been the route by which the virus gained access to the brain. Considered from the standpoint of the severity of the lesions, it could well be argued that the primary site of attack was the cerebral cortex.

One other point is worthy of comment, and that is that various stains were employed in an effort to determine which was the most satisfactory in demonstrating the inclusion bodies, and best results were achieved with the standard hematoxylin and eosin method.

TRANSFER OF IMMUNITY TO THE VIRUS OF ST. LOUIS ENCEPHALITIS TO SUCKLING MICE THROUGH THE MILK, DEMONSTRATED BY FOSTER NURSING. Margaret G. Smith and (by invitation) Betty B. Geren, St. Louis, Mo.

Abstract. Four groups of suckling mice 7 to 12 days of age, a total of 217, were used in this experiment. Two groups were mice born of mothers immunized by two intraperitoneal inoculations of the St. Louis encephalitis virus. The second intraperitoneal inoculation had been given 3 weeks prior to mating. The other two groups were mice born of non-immunized mothers. One group of mice born of immunized mothers was transferred at birth to non-immunized mothers for foster nursing. Similarly, a second group of mice born of non-immunized mothers was transferred at birth to immunized mothers. The two other groups of mice, one born of immunized and one of non-immunized mothers, were allowed to remain with their own mothers. The suckling mice were tested for resistance by intraperitoneal inoculations of the virus. The two groups of mice nursed by immunized mothers showed significantly greater resistance to the virus than did the two groups nursed by non-immunized mothers. The latter two groups showed no significant difference in resistance to the virus, although one group was born of immunized mothers and the other group of non-immunized mothers.

MYOCARDIAL LESIONS IN POLIOMYELITIS. Vera B. Dolgopel and (by invitation) Mary D. Cragan, New York, N.Y.

Abstract. Acute myocarditis has been found at necropsy in 16 of 87 cases of poliomyelitis. The ages of patients ranged from 13 months to 37 years. The foci of myocarditis were small, occupying only a small portion of a low-power microscopic field. Several lesions were usually found in the same section, but in multiple blocks from a heart lesions were rarely found in more than 2 blocks. The

posterior wall of the left ventricle and the posterior papillary muscle were the sites most frequently affected.

Lesions of three types were encountered. In the first type, which at low power showed only increase in the number of nuclei, individual muscle fibers were thin and cloudy, with long, wavy nuclei clinging to them. In the second, cellular collections, predominantly mononuclear, were present between myocardial fibers which usually were intact. The third type consisted of collections of cells in the interstitial tissue around the blood vessels. The cells usually were histiocytic mononuclear cells, but in one case the infiltrate consisted of polymorphonuclear leukocytes and lymphocytes.

Pneumonia was present in 4 cases; 3 cases showed minimal inflammatory lesions in the lungs, 9 showed no pulmonary inflammation. In 4 cases death was apparently caused by cardiac failure.

The incidence of myocarditis was 18.4 per cent in the entire material. However, in cases with one section available for examination the incidence was 9.3 per cent, and in 44 cases with multiple slides it was 27.2 per cent. The latter figure is probably closer to the actual incidence of myocarditis in fatal poliomyelitis.

CARDIOVASCULAR LESIONS IN ACUTE POLIOMYELITIS. Ted E. Ludden (by invitation) and Jesse E. Edwards, Rochester, Minn.

Abstract. By means of the method of Gross, Antopol, and Sacks for histologic examination of the heart, myocarditis was demonstrated in 14 (40 per cent) of 35 cases in which acute poliomyelitis was fatal. Myocarditis was classified as severe in 6 cases, moderately severe in 4, minimal in 3, and healed in 1. In those hearts classified as demonstrating severe myocarditis, focal coagulation necrosis of myocardial fibers and associated infiltration of neutrophils were conspicuous manifestations. The principal histologic evidences of myocarditis in the other specimens were perivascular collections of large mononuclear cells and minimal alterations of myocardial fibers. In the heart classified as "healed" there were focal lesions characterized by the complete disappearance of myocardial fibers; in these areas only myocardial stroma remained.

Three patients who had myocarditis presented distinctly unusual findings. In one there was rupture of the myocardium of the right atrium. In another there was acute vegetative endocarditis of the mitral valve, and in a third there was acute vegetative endarteritis of a patent ductus arteriosus.

Since animal inoculations were not carried out in this study, the relationship of the poliomyelitis virus to the observed lesions was not definitely determined. However, the high incidence of myocarditis among this group of patients and the frequency of myocarditis reported by Saphir and others would seem to indicate that in the presence of poliomyelitis myocarditis is actually due to the virus of poliomyelitis.

*Discussion of Papers by Drs. Dolgopel and Cragan, and
Ludden and Edwards*

(Dr. Jacob Werne, Jamaica, N.Y.) I believe it should be emphasized that these lesions are not at all specific. Similar lesions were described some time ago by Parker and Nye as tissue reaction to streptococcal infection. We have encountered these lesions frequently in association with fulminating infections. Their incidence in subjects dying with acute infection of whatever cause will vary with the extent of the histologic study.

(Dr. Kornel L. Terplan, Buffalo, N.Y.) In the post-mortem material of the large epidemic of poliomyelitis in 1944 in Buffalo, in the cases examined (about 40

of 60), only few minimal interstitial infiltrations of focal character were found in sections taken from the heart. In one case they were somewhat more marked. In about 15 cases the heart was not examined. Usually one section was taken from the right, and one from the left ventricle. The fatal cases in that epidemic were practically all of the bulbar type. In all, acute inflammatory conditions of the respiratory tract were observed, and it appeared to me that these lesions pointed to a failure of peripheral respiration as the immediate cause of death. In the pathogenetic consideration of the lesions found in the heart muscle, the inflammatory changes found in the lungs and in the upper respiratory tract should be considered as a possible factor.

(Dr. Dolgopel) I think I will agree with Dr. Werne that the lesions are probably not characteristic of poliomyelitis alone, and that this is one of those myocarditides observed in many infectious diseases.

As to pulmonary infections being the cause of myocarditis in our cases, 9 of the 16 cases showed no inflammation in the lungs or bronchi. We had a number of blocks from the lungs available for examination, and there was no inflammation in these 9 cases. There was pneumonia in 4, and only minor evidences of polymorphonuclear infiltration in a few alveoli in 3 others, so I would say that the majority of our cases were free of pulmonary infection.

(Dr. Edwards) Pneumonia was not a common factor in our cases. Of the 14 patients with myocarditis, 3 had pneumonia.

As to the implication that this lesion is caused by a virus, let me say that it is difficult to determine whether a virus is a direct infecting agent in a lesion of this kind. Even if a virus were isolated in the myocardium, it might be difficult to ascribe lesions directly to the virus. In poliomyelitis, myocarditis is something with which one must deal and I would like to say in reference to the recent study of Parker and his associates of cases in which the influenza virus was identified in the body, that they found myocardial lesions which were identical to those which we found in these cases of poliomyelitis.

OBSERVATIONS WITH IMPROVED ELECTRON MICROSCOPIC TECHNIQS ON THE INTERNAL STRUCTURE OF *ESCHERICHIA COLI* CELLS AND THE GENERATION OF COLIPHAGE. James Hillier (by invitation), Stuart Mudd and (by invitation) Andrew G. Smith, Philadelphia, Pa.

Abstract. New lenses, objective apertures, and preparative technics have made it possible to begin investigation of the internal structure of bacterial cells. In electron pictures of normal *Escherichia coli*, strain B, the fine structure of the protoplasm is resolved as particles spaced in three dimensions; linear aggregation is apparent in thinner regions of the protoplasm. Adjacent to the ends of the cells and to the planes of division the protoplasm is relatively dense. Between these dense areas appear regions of low density containing granules and rodlets of very dense matter; these correspond in position and appearance with the chromatinic bodies described by C. F. Robinow.

Coliphage T₂ may cause lysis of *Esch. coli* B cells without phage particles being detectable. Adsorption of phage particles to *Esch. coli* B cells is followed by reorganization of the cell contents. The fine structure of the protoplasm becomes coarser. Bacteriophage particles appear throughout the cell protoplasm, often in linear aggregates aligned in an interparticle matrix; the appearance of the individual particles varies from those in which structure is clearly defined to those in which definitive structure is suggested only. When *Esch. coli* cells are lysed, certain evidences of pattern may be discernible in the débris. For instance, the cell wall yields many characteristic elliptical and circular segments. In older

preparations cells may be found packed with phage particles. Our observations are in better accord with the conception that the coliphage particles are synthesized by an altered metabolic mechanism of the parent cell than that they multiply by fission or other means.

Discussion

(Dr. Edwin W. Schultz, Stanford University, Calif.) We have been working with another phage, a pyocyanus bacteriophage, and it has been our observation also that the particles of this phage are always of much the same size and we have never been able to observe anything in our micrographs suggesting that they undergo division. However, I wonder how Dr. Mudd would explain the intricate structure, within the head portion, for example, on the basis of an auto-catalytic type of reproduction? It seems to me that this is too complex to be easily explained on such a basis.

(Dr. Stanley H. Durlacher, Edgewood, Md.) Dr. James S. Murphy and I performed a very crude experiment. We thought it might be interesting to take *Esch. coli*, extract them with various fat solvents, acids and alkalis, and study them with the conventional electron microscope. We did this and I believe it was our alkali-treated organisms that showed an inhomogeneity of structure which resembled very much that shown by Dr. Mudd with the improved electron microscope. I wonder whether he has ever done anything of this sort, and whether we are seeing the same thing he did.

(Dr. Mudd) In answer to Dr. Schultz' question. I think that the earlier hypothesis of autocatalytic generation of bacteriophage from precursors already present in the host cell is quite out of accord with many facts which are currently available. On the other hand, I am impressed with two manuscripts by Seymour S. Cohen, at present in press in the Journal of Biological Chemistry, which indicate that infection with coliphage radically diverts the metabolism of the parent cell from the elaboration of *Esch. coli* protoplasm to the synthesis of desoxyribose nucleoprotein characteristic of the infected phage. P₃₂ present as phosphate in the external medium was shown to be the principal source of phosphorus for the desoxyribose synthesized. In accord with this our electron microscopic pictures seem to us in much better agreement with the conclusion that phage particles are synthesized in the altered protoplasm of the parent cell than that they result from division of phage particles.

MUCORMYCOSIS, WITH REPORT OF ACUTE MYCOTIC PNEUMONIA. Roger D. Baker and (by invitation) A. O. Severance, Birmingham, Ala., and San Antonio, Texas.

Abstract. A case of acute lobular pneumonia in a child of 3 years in Texas, due to *Mucor*, is reported. Mention is made of another case of mucormycosis of the nares and brain. As 4 cases of mucormycosis have recently been reported in the American literature, 6 cases are available. All have been acutely fatal and have occurred in patients with diabetes mellitus. The fungus produces necrosis and acute inflammation, grows in blood vessel walls, and causes thromboses. A brief summary of the older, chiefly German, literature on mucormycosis is presented.

Discussion

(Dr. Alfred Golden, Memphis, Tenn.) We observed and reported the 3 cases to which Dr. Baker referred, and it seems to me this peculiar infection in the diabetic offers us another tool in the differential diagnosis of non-pathogenic and

pathogenic fungi. We were impressed with the invasiveness of this organism, as Dr. Baker was. We observed in 2 of the cases that the organism could invade tissues as tough as the sclera, and in some cases produced a polymorphonuclear response, and in others it did not. It is commonly assumed that the degree of leukocytic reaction to a fungus is an index of pathogenicity, but I think we have another lesson to learn from Dr. Baker's case, as well as from the others: that we should pay more attention to the invasiveness of a fungus, even if there is little tissue reaction around it.

MELIOIDOSIS. REPORT OF SECOND CASE FROM THE WESTERN HEMISPHERE, WITH BACTERIOLOGIC STUDIES ON BOTH CASES. Parker R. Beamer, and (by invitation) Philip L. Varney, Wilson G. Brown, Frank McDowell, and Birkle Eck, St. Louis, Mo.

Abstract. Melioidosis is a specific, glanders-like infection in human beings, caused by a small, pleomorphic, Gram-negative, rod-shaped bacterium, which resembles the glanders bacillus in some respects while differing from it in other characteristics. In 1912 Whitmore first isolated the causal agent from human cases of melioidosis in Rangoon. Although the organism is classified as *Malleomyces pseudomallei*, it is known also under other names such as *Bacillus pseudomallei*, *B. whitmori*, *Flavobacterium pseudomallei*, *Pseiferella whitmori*, *Actinobacillus pseudomallei*, and *Loefflerella whitmori*.

Ordinarily, melioidosis is an acute pulmonary infection, with hematogenous dissemination of the organisms to several viscera, producing numerous miliary abscesses, and septicemia followed by death in a few days. Some 300 cases are recorded in the medical literature, approximately two-thirds of these occurring in Rangoon, and the remainder in other areas in the Far East. Only a few cases of chronic melioidosis have been encountered, and usually in individuals who survived the acute form of melioidosis. With one exception all of these patients were believed to have contracted the disease in the endemic region described above.

McDowell and Varney reported a case of chronic melioidosis, believed to be the first one which originated in the Western Hemisphere. This patient had not been out of the United States except for 2 years in Panama, several years prior to the onset of the disease.

The present report concerns a 25-year-old woman member of the U.S. Marine Corps admitted to the hospital with chief complaints of soreness and swelling in the right lower abdomen. Examination revealed tenderness and induration in the right inguinal region. Subsequently this area desquamated, exposing deeper layers of the skin partially covered by serofibrinous exudate. Several tender nodes became apparent in both inguinal regions, and, from time to time, vesicles and bullae developed in the skin. Gradually the lesions in the skin spread to the left inguen. Microscopic examination of a biopsy from this region revealed numerous inflammatory cells in the subcutaneous tissues, chiefly lymphocytes and plasma cells with a few polymorphonuclear leukocytes, but no evidence of a specific causal agent. The skin lesion continued to spread, the patient's general condition grew steadily worse, and she died approximately 9 months after admission, after failure to respond to intensive treatment with sulfadiazine, penicillin, streptomycin, fuadin, and other therapeutic measures.

Post-mortem examination revealed a large chronic abscess, underlying the skin lesions described above and involving the retroperitoneal tissue with necrosis of subcutaneous structures and muscle of the left side up to the perirenal area, posteriorly across the midline to the right side, and downward into the muscles

and subcutaneous tissues of the left thigh. Microscopically, the wall of the abscess was composed of an outer layer of slightly or moderately dense fibrous tissue with several thick-walled blood vessels and numerous inflammatory cells, chiefly lymphocytes, plasma cells, and a few polymorphonuclear leukocytes. The inner portion of the abscess wall was comprised of partially organized fibrinous exudate containing numerous polymorphonuclear leukocytes. Microscopic examination of the liver revealed dissociation of hepatic cords in an advanced degree, necrosis of hepatic cells, and several minute foci of granulomatous inflammation. In the kidney several of the distal convoluted tubules were involved in focal granulomatous inflammation. These visceral lesions may represent the effect of sulfa drug, or they may be associated with the primary infection.

Portions of the wall of the abscess were cultured on blood agar and numerous colonies of poorly staining, Gram-negative, bipolar, motile, pleomorphic rod-shaped bacteria were isolated, in smooth and rough forms. Morphologically, the organisms resembled the glanders bacillus, with the exception of motility, and the colonies resembled those of *M. pseudomallei* described in the literature. After thorough study of morphologic and biochemical characteristics the organism from this case was identified as *M. pseudomallei*. It is almost identical with the strain isolated from the first case. Each organism was agglutinated by the respective patient's serum to a significant titer, and by antiserum prepared with a known strain of *M. pseudomallei*. Complete bacteriologic studies will be reported in a paper now in preparation.

Discussion

(Dr. Joseph F. McManus, Birmingham, Ala.) Were animals inoculated with this material? Mice, I think, are the most important.

(Dr. Beamer) Mice and guinea-pigs were inoculated with the organism isolated from both of these cases. Straus' reaction in a slight degree was observed in guinea-pigs inoculated intraperitoneally. Both strains of the organism were more virulent for white mice. Small amounts of young cultures produced death regularly. Post-mortem examination of infected animals revealed thick, viscid exudate in the peritoneal cavity. Organisms were present in large numbers in this exudate and also in the blood. If animals survived beyond a few days, small focal lesions were noted in the viscera. Cultures from these organs, particularly from the focal lesions, resulted in isolation of the organism.

CYTOPLASMIC INCLUSION BODIES IN INTESTINAL EPITHELIUM OF MICE. RELATION TO DIARRHEAL DISEASE IN SUCKLINGS. Alwin M. Pappenheimer and (by invitation) F. Sargent Cheever, Boston, Mass.

Abstract. In a previous report, Pappenheimer and Enders described the occurrence of intranuclear inclusions in a large proportion of suckling mice with diarrhea. The original stock in which the disease had been prevalent for several years was accidentally destroyed during the summer of 1946. Since then, new stock from several sources has developed diarrheal disease clinically indistinguishable from that occurring in the original stock. In no instance has it been possible to demonstrate intranuclear inclusions of the type described. However, cytoplasmic inclusions, best shown with the Laidlaw acid fuchsin-orange G stain, are present in a high proportion of the suckling mice with diarrhea arising either spontaneously or following the oral administration of extracts of diarrheal intestine. They are limited to the epithelial cells of the small intestine, over the summits of the villi, and are regularly present during the first few days of

the disease. Later, the inclusion-bearing cells are desquamated into the lumen. There is no inflammatory reaction. Normal stock mice, or controls fed with boiled extract, or extract of intestine from normal mice, do not show cytoplasmic inclusions of this type.

Discussion

(Dr. William H. Carnes, Baltimore, Md.) I would like to ask Dr. Pappenheimer whether he has ever looked for similar inclusions in the intestines in cases of infantile diarrhea, and if so, whether he has seen anything that resembles these.

(Dr. Pappenheimer) I regret very much that I have not had opportunity to study human material. One has to make sure of proper fixation, because the superficial epithelium is so easily desquamated after death, and that is where one finds the inclusion bodies in the mouse intestine. Furthermore, if there is any analogy to the mouse diarrhea, one would expect to find inclusions only in the first days of the disease.

READ BY TITLE

THE BLOOD AND BONE MARROW IN PATIENTS WITH CIRRHOSIS OF THE LIVER.

Lawrence Berman and (by invitation) Arnold R. Axelrod, Detroit, Mich.

Abstract. The peripheral blood and bone marrow findings in cirrhosis of the liver have been analyzed on the basis of a review of the literature and the authors' study of 25 patients with diagnoses verified by biopsy of the liver. The principal blood findings are macrocytic or normocytic anemia with normal or elevated mean corpuscular hemoglobin values, lymphopenia, and thrombocytopenia in the majority of the cases. Anemia may be independent of bleeding, and the severity of the anemia or macrocytosis does not appear to be related to the severity or duration of the liver lesion in patients with cirrhosis, although this appears to be true of experimental cirrhosis in rats. The consistent change in the bone marrow is extension of the marrow organ so that active hematopoiesis is found in the shafts of the long bones. Regardless of the presence or absence of bleeding or anemia, the marrow of the sternum is of normal or increased cellularity, with normal or increased erythrocytogenesis and megakaryocytogenesis in most cases. Hypocellularity of the marrow is an unusual finding, even in patients with advanced liver lesions. Macronormoblastic erythropoiesis is seen in patients with macrocytic anemia, but megaloblastic erythropoiesis does not result from hepatic cirrhosis.

The presence of peripheral cytopenias (anemia and thrombocytopenia), in spite of normal or increased formation of erythroblasts and megakaryocytes in the marrow, is suggestive of hypersplenism in patients with cirrhosis of the liver. In patients with chronic hemorrhage the blood and sternal marrow pictures are those of iron-deficiency anemia, although other changes such as lymphopenia and thrombocytopenia tend to persist.

The combined studies of the peripheral blood and sternal marrow are often of value in establishing a diagnosis of cirrhosis of the liver.

SEX DIFFERENCE IN THE ALKALINE PHOSPHATASE DISTRIBUTION IN THE KIDNEY OF THE MOUSE. Thelma B. Dunn, Bethesda, Md.

Abstract. A sex difference was noted in the distribution of alkaline phosphatase in adult male and female kidneys from 5 inbred strains of mice. In both the male and female, the cells of a short segment of the proximal convoluted tubules after leaving the glomeruli were intensely stained throughout. In the male, an

additional segment showed an intense staining of the brush borders only. This difference developed with sexual maturity, and it was not detected in immature mice or in other species examined, namely, the rat, guinea-pig, and rabbit.

THE EFFECT OF SULFATHIAZOLE UPON EXPERIMENTAL PYELONEPHRITIS IN RABBITS. John H. Fisher and (by invitation) N. O. Toplack, London, Ont.

Abstract. Not received.

A HISTOLOGIC AND CHEMICAL STUDY OF NECROSIS OF SKELETAL MUSCLE IN ACUTE ISCHEMIA. John W. Harman and Rodney P. Gwinn (by invitation), Madison, Wis.

Abstract. It was previously observed that after the institution of complete ischemia in the limbs of rabbits and rats a progressive, characteristic necrosis commenced to manifest itself at the fourth hour with the appearance of Bowman's discoid degeneration. A further study revealed that release of the major vascular occlusion failed to preclude extension of the necrosis because of the resultant stasis. In a closely correlated study of the histologic and biochemical changes and the contractility in such muscles, it is found that the extent of the necrosis reaches its maximum by 3 hours subsequent to release of the tourniquet and is proportional to the duration of the ischemia. Contractility rarely reappears until a lapse of 20 hours after release of the tourniquet and the percentage of muscles which are excitable is also dependent upon the duration of the ischemia and the extent of the necrosis.

In muscles ischemic for 4 hours it is known that the high-energy compounds, glycogen, adenylypyrophosphate, and phosphocreatine, are completely hydrolyzed and are not resynthesized within 4 hours of release of the tourniquet. This is substantiated by inability of such muscles to contract upon faradic stimulation. After 24 hours, however, over 80 per cent of such muscles are contractile to faradic stimulation. Chemical analysis reveals that in these there is a significant resynthesis of glycogen, adenylypyrophosphate, and phosphocreatine. Lactic acid also is present in greater quantity than in normal muscle.

It is suggested that the anoxia associated with the ischemia permits depletion of the high-energy reserves, which initiates the collapse of the cellular structure, as indicated by the proportionality of this to the duration of ischemia and by the rapidity with which this is reached after relief of the ischemia. On the other hand, the very considerable structural survival and both biochemical and physiologic recovery of muscle fibers accord most aptly with the view that cell death and "biochemical irreversibility" are not dependent upon depletion of the energy reserves unless this is associated with some more profound alteration. It is believed that this determinant of irreversibility is the structural disintegration which, though the final event in the sequence, is the decisive one. This structural change is seen in several forms to which are applied the designations of Bowman's discoid degeneration, Zenker's hyaline degeneration, and Fishback's granular degeneration, even though all have a similar pathogenesis.

HODGKIN'S GRANULOMA INVOLVING BONE. John B. Hazard, Cleveland, Ohio.

Abstract. Bone involvement in Hodgkin's granuloma at autopsy is rather common, but as a presenting lesion, especially without detectable changes in the peripheral lymph nodes, it is unusual. Recently 2 cases in the latter category were observed. A woman, 47 years of age, with pain and a mass in the sacro-iliac region, on roentgenographic examination, was found to have an osteoblastic lesion of the left ilium and also evidence of a pulmonary mass with enlarged

mediastinal lymph nodes. Biopsy of the ilium revealed typical Hodgkin's granuloma. A man, 45 years old, with the complaint of pain in the chest and back, roentgenographically presented an expanded, rarefied lesion in the 8th rib. The resected segment was replaced almost entirely by semifluid yellowish tan tissue forming an ovoid mass 4 cm. in diameter, with an irregular bony shell. Unlike the usual lesion of Hodgkin's disease, the mass was formed principally by purulent exudate, often in large lakes, and by granulation tissue. The latter was infiltrated by many polymorphonuclear neutrophils, in patches by eosinophils and lymphocytes, and by large mononuclear cells, which were in considerable numbers in some locations and occasionally presented atypism evidenced by variability in size and nuclear vesicularity. The lesion was regarded as a granuloma, probably eosinophilic granuloma. Subsequently, the axillary and inguinal lymph nodes became enlarged and on biopsy were typical of Hodgkin's disease. Though Hodgkin's granuloma has not been authenticated as arising primarily in bone, the osseous lesions may be a primary manifestation of considerable importance in diagnosis of the disease. Softening and suppuration may be prominent pathologic features.

THE TOPOGRAPHY OF CHRONIC GASTRITIS IN OTHERWISE NORMAL STOMACHS.*

Robert Hebbel, Minneapolis, Minn.

Abstract. Ninety-seven stomachs, free of ulcer, scar, tumor, and obscuring post-mortem changes, obtained at autopsy from individuals of all ages and both sexes, whose past histories recorded no gastric complaints, were searched in sections of rolls of mucosa from the entire lesser and greater curvatures, respectively, for evidences of chronic gastritis. The presence, severity and distribution of lymphocytic infiltration, lymph follicles, atrophy, intestinal metaplasia, pseudopyloric glands, cysts and erosions were recorded. Any parenchymal lesion was considered abnormal. Lymphocytic infiltration in excess of mild degree was considered abnormal, but those specimens which showed only excess infiltrate were kept separate. The changes were noted to be focal (isolated parenchymal lesions well within a microscopic field), patchy (several somewhat larger lesions), or diffuse (involving the whole segment considered).

Abnormality in some degree was found in 70 (72 per cent) of the specimens. Of the 27 stomachs free of changes, 20 were from persons less than age 51 and 7 were from those over age 50. There was no uniformity of involvement between antrum and body, and in either segment the changes varied from isolated foci to diffuse alterations. Some specimens showed focal or patchy lesions on one or both curvatures. A few showed diffuse changes on one curvature and focal or patchy lesions on the other. Some showed diffuse changes on both curvatures, but these made up a small proportion of the total. Many of the lesions were not quantitatively significant but where, short of diffuse involvement, to draw a line between normal and abnormal on the basis of quantitative change is uncertain.

The antrum and body are best considered separately. The antrum was abnormal in some degree in 64 specimens (66 per cent of the total, 50 per cent of the 44 specimens from persons less than age 51, 89 per cent of the 53 specimens from those past age 50). Focal lesions predominated in the younger group. Diffuse parenchymal changes were found in 8 specimens (8.2 per cent of the total), i.e., in 1 (2.3 per cent) of those from persons under age 51 and in 7 (13 per cent) of those from persons over age 50.

The body mucosa was abnormal in some degree in 48 specimens (49.5 per

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

cent of the total, *i.e.*, 27 per cent of those less than age 51, 68 per cent of those over age 50). Diffuse parenchymal changes were found in 14 specimens (14.4 per cent of the total) and thus occurred in 3 (6.8 per cent) of those less than age 51 and in 11 (20 per cent) of those over age 50. Diffuse parenchymal changes involving both antrum and body were observed in but one specimen.

THE EFFECT OF LOW PROTEIN AND LOW CHOLINE DIETS ON THE ABSORPTION OF IRON AND COPPER. D. M. Hegsted (by invitation), T. D. Kinney and J. A. Cartaya (by invitation), Boston, Mass., and Cleveland, Ohio.

Abstract. Rats were fed on a diet low in protein (8 per cent casein) and choline but adequate in vitamins and minerals, to which was added 2.2 per cent ferric citrate or 0.1 per cent copper sulfate, or the two salts together. Iron was definitely toxic as judged by the gross appearance, survival time, and weight loss. Copper was not toxic, but the combination of iron and copper was more toxic than iron alone. Choline did not protect against this toxicity, but the further addition of protein prevented all evidence of toxicity. The nature of the toxic action of iron is not clear. The iron content of the livers was slightly elevated and some iron could be demonstrated in the liver cells on histologic examination, but it is believed that this was not great enough to be the cause. Lack of phosphate absorption was apparently not involved since there was no significant difference in the amount of bone ash between animals receiving iron and control animals. Examination of the bone also showed no variation from the control animals.

Choline and copper appeared to be interrelated. Copper largely prevented fat deposition in livers of rats on choline-deficient diets. Further, when choline was added to the diet larger quantities of copper were present in the liver.

THE PATHOLOGIC EFFECTS OF TWO PHOSPHINE OXIDE ANTICHOLINESTERASES. H. Walter Jones, Jr., and Benjamin Landing (by invitation), Bethesda, Md.

Abstract. During investigation of the relationship between the toxicity of organic phosphorus compounds and their inhibition of brain cholinesterase *in vitro*, it was discovered that p-chlorophenyl diethoxy phosphine oxide (A) and bis (p-chlorophenyl) ethoxy phosphine oxide (B) significantly inhibit cholinesterase, A being over ten times as active as B. However, deaths resulting from a single injection of either compound frequently were delayed as much as 5 days, whereas the anticholinesterases di-isopropyl-fluorophosphate and tetraethyl pyrophosphate cause immediate deaths only. Pathologic effects following intraperitoneal administration of these compounds were therefore studied in mice, rats, dogs, and rabbits.

The lesions produced by both compounds in the various organs were essentially the same in all animals. The splenic follicles showed pyknosis and degeneration of lymphocytes, and phagocytosis of debris by large reticulum cells; larger doses produced hypocellularity without phagocytosis in both white and red pulps. After 2 days the mitosis rate in the follicles tended to increase. The thymus showed widespread pyknosis and degeneration of the cortical lymphocytes. The changes produced in lymph nodes and Peyer's patches consisted of lymphocytic degeneration and phagocytosis of debris after smaller doses, and more marked lymphocytic degeneration and hypocellularity without phagocytosis after larger doses. Congestion and mild hypocellularity were the only changes observed in the bone marrow.

In mice, the superficial protoplasm of the renal proximal convoluted tubules sloughed into the lumina, forming protein casts. In rats, this cytoplasmic slough

did not occur, but the nuclei of the epithelial cells of the proximal convoluted tubules were often pyknotic. In the gastrointestinal tract, increased mucous secretion and an increased number of mitotic figures in the epithelium of the glands were observed. In the testis, pyknosis of all of the spermatogenic layers with shrinking or loosening of the layers was the only change observed. In a few animals with relatively severe lymphoid damage, the Kupffer cells of the liver were pyknotic. The lungs during the first 48 hours showed moderate atelectasis, with increased leukocytes in the alveolar capillaries, and congestion. The brain after B showed mild edema and shrinkage of some cortical ganglion cells. Peripheral (sciatic) nerve was examined in one dog after three injections of B over a period of 20 days. No evidence of peripheral neuritis was observed. Compound A produced microscopic evidences of peritonitis, perisplenitis, and peripancreatitis; these did not occur with B.

The total and differential leukocyte count were unaffected by single LD₅₀ doses of A or by six injections of one-half LD₅₀ each during an 8-day period, but doses of 2.5 LD₅₀ caused an absolute lymphopenia and usually a polymorphonuclear leukocytosis.

The sulfur present in the urine as ethereal sulfate was elevated after injections of A in rats and rabbits, suggesting that these compounds are excreted as sulfate conjugates.

Visceral lesions of the type described are not observed following lethal doses of more active anticholinesterases, so that these compounds may have some other mode of action. The pattern of organs damaged, lymphoid tissues, testis, kidney, and gastrointestinal tract, suggests that seen with mustards, but the effects are much less severe than those produced by many mustards at comparable dose levels and seem inadequate to account for death. The doses of these two compounds necessary to damage proliferating tissues are so toxic that they appear to have no potential use in tumor therapy.

DEVELOPMENT AND PATHOGNOMONIC EVALUATION OF GIANT CELLS IN BONE TUMORS AND SIMILAR CONDITIONS. Fritz Levy, Huntington, W. Va.

Abstract. The term "giant cell" is used for two different formations which are remarkably larger than the average or standard cell of a certain tissue. The first one is characterized by unsharp or sharp limitation of the cytoplasm and a number of nuclei of the same size. This formation is represented in normal tissue by the so-called osteoclast. The second type includes polyploid cells with 1 or more nuclei with increased chromosome number; it is represented in normal tissue by the megakaryocyte of the bone marrow.

Both kinds of formations are found in various neoplastic diseases. They do not have special functions, but, if they have any functions at all, these are remainders of the functions of the cells of origin. The different ways of development show that only the presence of numerous polyploid cells is an essential factor in the diagnosis of malignancy, since single polyploid cells occur occasionally in all tissue.

BRUCELOTIC OSTEOMYELITIS OF THE SPINAL COLUMN IN MAN. Leo Lowbeer (by invitation), Tulsa, Okla.

Abstract. It is well recognized that *Brucella* organisms not only cause septicemia but also focal inflammations, often of granulomatous character, in man and other animals. Osteomyelitis of the spine and other bones of the hog caused by *Brucella suis* has been described by Feldman, Graham, and others, and I have

studied its histologic character. Osteomyelitis can be produced in about 30 per cent of *Brucella*-inoculated guinea-pigs. In man approximately 200 cases of brucellic osteomyelitis have been described by roentgenologists and clinicians, three-fourths of which showed involvement of the spine. These were caused predominantly by the melitensis strain. The low mortality of brucellosis partly accounts for the almost complete absence of microscopic studies on brucellic osteomyelitis.

Through the courtesy of Dr. T. de Villafane Lastra, Professor of Epidemiology at the University of Cordoba, Argentina, I have been fortunate enough to obtain three human spines from patients who died with subacute brucellosis, melitensis type, and who had developed clinical symptoms of spondylitis. The gross specimens showed small and large areas of destruction of disks and contiguous vertebral bodies in the dorsolumbar spine. Occasionally, small osteomyelitic foci were found in anterior or central portions of vertebrae. Exostoses were frequent. Microscopic examination showed subacute osteomyelitis with destruction of cancellous bone, end-plate, cartilage-plate and disk by granulation tissue which in early phases consisted of polymorphonuclear leukocytes, lymphocytes, and plasma cells, and enclosed small abscesses, the contents of which have a tendency to become necrotic. In this phase the process is similar to that found in pyogenic osteomyelitis of the spine, but less destructive, less purulent, and perhaps with more tendency to repair. It also resembles experimental osteomyelitis produced in *Brucella*-inoculated animals.

In later stages large mononuclear cells, fibroblasts, and occasional bizarre multinucleated giant cells of foreign body type appeared. Still later the granulation tissue consisted of large macrophagic histiocytes, surrounded by thick layers of lymphocytes and plasma cells which in turn were surrounded by young fibroblasts and connective tissue. It was well vascularized but underwent extensive central coagulative necrosis of perhaps allergic origin. It contained large numbers of multinucleated giant cells of foreign body type, apparently osteoclastic in nature, surrounding or enclosing small bony sequestra. There was also formation of tubercle-like granulomatous nodules composed of histiocytes of non-epithelioid appearance. Acid-fast bacilli could not be demonstrated. In this phase the process resembles *Br. suis* osteomyelitis of man as described by me, and also spontaneous brucellic osteomyelitis of the hog. Necrosis, therefore, occurs in melitensis as well as in *suis* infections.

Whether or not these lesions are actually caused by *Brucella* cannot be stated with absolute certainty as long as cultures taken directly from the affected vertebrae are not available. Since, however, these lesions occurred in cases of active subacute brucellosis with positive blood cultures; since, in the absence of fistulae, there was no likelihood of secondary bacterial invasion; since the absence of a truly suppurative inflammation in the early phase speaks against infection by pyogenic organisms; and since the later granulomatous-necrotizing phase bears no true resemblance to tuberculous infection, one may safely assume that the lesions are brucellic in character.

PATHOLOGY OF RUPTURE OF THE SPLEEN IN ACUTE VIVAX MALARIA. Joseph M. Lubitz, Wood, Wis.

Abstract. A pathologic study was made of the ruptured spleen in acute vivax malaria. Four cases were available for examination, in 3 of which *Plasmodium vivax* was isolated and in the fourth the diagnosis of malaria was highly probable. Rupture of the spleen in malaria is preceded by a subcapsular hematoma. Char-

acteristically, the microscopic picture is that of diffuse reticulum cell hyperplasia, subendothelial and adventitial leukopoiesis, and dilatation of sinusoids and venules. Thrombosis and infarction may occur, but were not found consistently. In all cases there was subcapsular dilatation of vessels with small hematomata, not only at the site of rupture, but also in distant areas. Extension of such hemorrhages to the capsule and their confluence appeared to produce the subcapsular hematoma. Stagnation of blood in the sinuses is believed to be caused by the general cellularity and the narrowing of the lumina by subintimal leukopoiesis. As stated by Rigdon, this is a contributing factor in the formation of thrombosis and infarction. Clinically, a pleural effusion on the left side may indicate splenic rupture.

THE STRUCTURE OF THE RENAL GLOMERULUS IN THE NORMAL HUMAN KIDNEY AND IN SOME DISEASE CONDITIONS.* J. F. A. McManus, Birmingham, Ala.

Abstract. 1. The basement membrane in the normal glomerulus as shown with the periodic acid-Schiff's reagent technic derives from Bowman's capsule and attaches to the arterioles at the glomerular root. It encloses capillary loops and certain infrequent stroma cells of the mesangium, and the latter in the intercapillary or axial space. 2. The axial space is prominent in diabetes mellitus. It appears finely fibrillar. 3. Marked involvement of the axial space can be seen in various disease conditions: acute inflammation in acute glomerulonephritis; glycoprotein accumulation in intercapillary glomerulosclerosis; vacuolation and reticulation in eclampsia; lipoid accumulation in "lipoid nephrosis."

FACTORS INFLUENCING COLLAGEN CONTENT IN EXPERIMENTAL CIRRHOSIS.* Thomas G. Morrione (by invitation), Burlington, Vt.

Abstract. Cirrhosis was produced in 220 rats by exposure to carbon tetrachloride vapors every other day for 35 days. The increase in hepatic collagen, as determined quantitatively by Lowry's method, followed a curve of exponential type. After stopping the carbon tetrachloride, significant decreases occurred in hepatic collagen. The decrease was greatest on a low protein diet supplemented with methionine, choline, and cystine. Little or no collagen resorption occurred on a low protein-high fat diet. Ligation of the portal vein in rats with cirrhosis interfered with resorption of collagen provided no adhesions were present. The latter favored reversal of the cirrhosis.

HISTOLOGIC LESIONS ENCOUNTERED IN SEGMENTAL ENTERITIS. Henry Rappaport and Fred H. Burgoyne (by invitation), and Hans F. Smetana, Washington, D.C.

Abstract. A detailed histopathologic study of 110 cases of segmental enteritis submitted to the Army Institute of Pathology between the years 1940 and 1947 revealed certain histologic features which could not be explained readily on the basis of ulceration and chronic inflammation alone. These were: (1) edema out of proportion to the severity of the inflammatory infiltration; (2) marked dilatation of the lymph vessels; (3) presence of granulomatous lesions having the morphologic features of non-caseating tubercles in the intestinal wall and in the regional lymph nodes.

Granulomas with giant cells were found in the intestines in 40 (36 per cent) of the cases and were of two main types. One was characterized by predominance

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

of giant cells of the foreign body type, did not appear well organized, and usually blended imperceptibly with the surrounding inflammatory reaction. The giant cells frequently contained foreign bodies of varied structure, the nature of which usually could not be identified. The second type of granuloma consisted of well organized, circumscribed masses of epithelioid cells, with or without giant cells, and arranged in a tubercle-like fashion, histologically indistinguishable from the non-caseating, tubercle-like lesion observed in sarcoidosis. This type occurred in the intestines in 21 (19.1 per cent) of the cases. Lymph nodes were available for study in 17 of these 21 cases and 16 of them showed identical non-caseating, tubercle-like granulomas. In none of these cases, however, was there clinical or autopsy evidence of generalized sarcoidosis. In comparison, none of the 22 autopsied cases of sarcoidosis on file at the Army Institute of Pathology showed any involvement of the jejunum or ileum.

An analysis of the racial distribution of segmental enteritis and sarcoidosis revealed the following:

| <i>Disease</i> | <i>White per cent</i> | <i>Negro per cent</i> |
|--|---------------------------|---------------------------|
| Segmental enteritis (all cases)..... | 94.4 | 5.6 |
| Segmental enteritis with non-caseating tubercle-like granulomas | 88.9 | 11.1 |
| Sarcoidosis | 39.4 | 60.6 |

Vascular changes were encountered in a considerable number of cases. Among these, marked endarteritis was seen most frequently. The intimal proliferation was sometimes confined to a limited segment of the vessel. In a few instances both granulomatous arteritis and phlebitis were observed. In some cases there was a peculiar type of granulomatous lymphangitis in which the granulomas encroached upon and obstructed the lumina of lymph vessels.

EXPERIMENTAL ENDOMETRIOSIS. Jacob M. Ravid, New York, N.Y.

Abstract. This problem was undertaken for the purpose of learning something about the pathogenesis of endometriosis. Forty rabbits were used. Pieces of endometrium were excised and then implanted in the skin, peritoneal cavity, ovary, and the anterior chamber of the eye. Some of the animals subsequently received injections of estrogens and of urine from pregnant women. After varying periods of time positive "takes" were obtained in about 60 per cent of the animals. Such lesions were nodular and cystic. Microscopically, these nodules were made up of islands of endometrium, some of which had the typical appearance of miniature uterine cavities. Some of them also showed proliferation of smooth muscle fibers and embryonal cytogenic stroma. These experiments demonstrate the comparative ease with which experimentally implanted bits of endometrium can be made to grow in the rabbit, thus lending support to Sampson's implantation theory of endometriosis.

OBSCURE AXILLARY LYMPH NODE METASTASIS IN CARCINOMA OF THE BREAST. Otto Saphir and (by invitation) George D. Amromin, Chicago, Ill.

Abstract. Axillary lymph nodes from 30 patients with carcinoma of the breast, which on routine examination were reported as uninvolved, were restudied histologically by means of serial sections. Of these 10, or 33.3 per cent, contained

carcinoma cells. No relationship could be established between a hyperplasia of the sinus endothelium or of the reticulum cells, or of so-called pre-invasive changes and the presence or absence of metastases in lymph nodes. The necessity of performing more careful and thorough examinations of nodes in regions of malignant tumors is emphasized. The only means of ruling out carcinoma metastases is examination by serial sections. These are essential for correct prognosis and evaluation of surgical results.

MORPHOLOGIC CHANGES IN SYPHILITIC LESIONS DURING THE HERXHEIMER REACTION. Walter H. Sheldon and (by invitation) Albert Heyman, Atlanta, Ga.

Abstract. The Jarisch Herxheimer reaction has long been recognized as a complication of the treatment of syphilis. This reaction is a unique phenomenon since it occurs only in response to antisyphilitic therapy. It is thought to be caused by the release of spirochetal breakdown products. The syphilitic lesions frequently show gross changes during this reaction, but no histologic observations have been reported.

We have made histologic studies of the cutaneous and mucosal lesions during the Herxheimer reaction in several groups of patients with secondary syphilis. Definite histologic changes occur during this reaction. These consist of congestion, edema, alteration of the vascular endothelium, and acute inflammatory cellular infiltration. The changes are confined strictly to the syphilitic lesions. They appear within 5 hours after treatment and subside within 18 to 24 hours. These histologic changes were found in practically all patients with clinical evidences of the Herxheimer reaction. Similar changes probably occur during the Herxheimer reaction in late syphilitic lesions of the cardiovascular and central nervous system.

Our findings show that histologic changes occur in syphilitic lesions during the Herxheimer reaction. These changes may account for the serious complications which are occasionally encountered. Our findings also reveal a similarity between the morphologic changes of the Herxheimer reaction and hypersensitivity of the tuberculin type. Further studies are in progress which may lead to a better understanding of some of the immunologic aspects of syphilitic infection.

THE PULMONARY MANIFESTATIONS OF SCLERODERMA: AN ANATOMIC-PHYSIOLOGIC CORRELATION. David M. Spain and (by invitation) Albert G. Thomas, New York, N.Y.

Abstract. Among the visceral changes occurring in scleroderma, those in the lung may lead to serious clinical results. The changes consist of hyaline fibrotic thickening in the interstitium of the lung parenchyma. Numerous cystic areas are present as well as compact areas of fibrosis. Many alveoli are gradually reduced in size. Associated bronchiolar changes give rise to patchy zones of obstructive emphysema. A case is presented with detailed respiratory and ventilatory functional studies. These studies are correlated with the necropsy findings. Both the respiratory and ventilatory functions are markedly impaired. The anatomic changes responsible are: Involvement of skin over the thorax with impairment of chest motion; fibrous contraction of the pleura with resultant compression of the lung; diffuse peribronchiolar fibrosis with obstructive emphysema; and diffuse interstitial fibrosis with impairment of gaseous exchange. At times these lung changes may be the first or the most prominent manifestation of scleroderma.

HETEROTOPIC BONE FORMATION IN THE SKIN. Robert E. Stowell, St. Louis, Mo.

Abstract. Nine instances of heterotopic bone formation in the skin are reported. Two occurred in basal cell carcinomas. Step sections through 40 healing or healed surgical scars revealed bone in 3 specimens. The findings in these cases, together with those in 100 similar cases collected from the literature, are discussed in considering probable etiologic factors in the formation of heterotopic bone.

DIFFERENTIAL DIAGNOSIS IN CONGENITAL SYPHILIS OF THE UMBILICAL CORD.*

Ruth C. Wanstrom and A. James French, Ann Arbor, Mich.

Abstract. Not received.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

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